

nature

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Facing up to demography

THE United States is gradually, if painfully, coming to grips with the problems of discrimination within its society. Most liberal-minded people have gone along with much that has been done in the past fifteen years, even when initial efforts to reduce discrimination have not been sophisticated or quick-footed enough to avoid charges of reverse discrimination, as in the case of Allan Bakke, a white student with good grades excluded from the medical school of the University of California at Davis. Racial minorities, women, the handicapped—these are causes with which very few would disagree. But scientists, in particular, are having very mixed feelings about the plight of those most recently claimed to be discriminated against—the elderly.

A bill that has just passed through the US House of Representatives with negligible opposition would prohibit mandatory retirement in private employment before the age of 70, and would prohibit mandatory retirement in the federal sector at any age (with exceptions such as the police and firefighters). At present private employers may retire their staff at 65, the federal government at 70. These moves came about for two reasons, both connected with demographic changes which are progressively peopling the United States with older citizens (23 million are 65 or over at present, and 31 million are expected to be so by the end of the century). First, the burden of social security payments is going up, yet the numbers of new workers is going down as the birthrate declines, so there are good actuarial reasons for wishing to postpone retirement. Second, the elderly are rapidly becoming a potent political force, of which politicians are becoming increasingly aware.

The bind for scientists is this: that deep concern was already being expressed before the retirement bill ever surfaced over the way that university faculty employment was moving towards a crisis. In 1979 there will be 4.3 million American eighteen-year-olds, 60% more than in 1960. The course of university expansion in the 1960s ensured that these young people would be well catered for. But after 1979, the numbers will steadily decline until in 1990 there will be fewer than 3.5 million in this age bracket. Many universities will presumably have to go out of business and elsewhere faculty will have to be trimmed. Things look particu-

larly bad in the physical sciences; an NSF projection puts the number of faculty positions in 1985 at 25% less than the number in 1972. Nor can this attrition be taken care of solely by retirement. Vigorous recruitment over the past thirty years has ensured that there is a predominance of young to middle-aged faculty members at present. So the prospects for the post-doctoral worker looking to get a toehold on the academic ladder already seemed bleaker than ever before. Nor should the position of the scientist working for the government be reckoned to be very different. Federal agencies have often followed very similar employment policies to those of the universities.

The new retirement legislation would obviously compound these problems very seriously, so it is small wonder that academic employers have been lobbying hard for exemption this past week. They have so far met with some success in that the Senate Human Resources Committee, in endorsing the bill (which now goes to the Senate), has excluded universities from its provisions. But no one seems yet to have spoken up for the federal government laboratories, where scientists will, presumably, be able to go on working to any age they choose.

Even if universities do emerge from the legislation relatively unscathed, there will still remain the urgent question of diminishing job prospects for the young. Richard Atkinson, Director of NSF, has recently made some fairly radical proposals (*Chronicle of Higher Education*, 28 March). These are that the government should facilitate more mid-career shifts for those who wish to depart early; that research institutes should be established close to or within universities to which senior academics could move, devoting more of their time to research and passing their teaching load on to newly employed junior faculty; and that industry should take greater advantage of the basic-research skills of senior academic scientists in some form of joint venture.

All of these proposals are doubtless open to many and varied criticism, but we simply cannot afford the luxury of a lengthy and hair-splitting debate on the subject. Action is needed in the very near future, otherwise demography will be upon us and we will be responding to the crisis in an arbitrary way. That would lead to an even worse form of discrimination. □



The long journey into the end of the artificial night

Sargun A. Tont, staff research associate at Scripps Institution of Oceanography, University of California at San Diego, offers a view on the study of solar eclipses

ALTHOUGH the occurrence of a solar eclipse is one of the most precisely predicted phenomena in the entire solar system, the chances of its coinciding with clear skies are about as good as Mr Buckley's inviting Mr Vidal for a transatlantic cruise on his yacht, or Bobby Fischer's opening with a queen's pawn.

Although unfriendly weather is an important obstacle for eclipse lovers, it is far away from being the only one. Getting to the path of an eclipse usually turns out to be a problem that itself exercises a good bit of human ingenuity. For the past of a total solar eclipse seldom favours highly populated regions. There is no reason that it should, of course, yet the number of eclipse paths which run toward far-away places almost appears designed to test the limits of human curiosity. Not surprisingly, then, the traditional vehicles for eclipse expeditions have been mules, donkeys, camels, sledges drawn by Alaskan huskies, trains and, of late years, even chartered jets. In 1972, a novelty was added: a huge, luxurious ocean liner, the *T.S.S. Olympia*, carried 800 eclipse freaks 900 miles out into the cold Atlantic.

The following paragraph from the *New York Times* of 30 July 1972 explains my presence on that ship of fools, as it appeared to me at the start:

A team from the Scripps Institute [sic] of Oceanography in La Jolla, California, would conduct an eclipse experiment possible only at sea; they would lower a 300-pound echo chamber over the *Olympia's* bow to determine whether the layer of microscopic ocean animals called plankton rises at night merely because it gets dark or because of an internal biological clock that would not be triggered by the extraordinary, sudden darkness of the eclipse.

In short, an eclipse provides a sort of artificial night for testing this unique hypothesis.

The ships may be more luxurious and larger, but the age-old problem of not being able to avoid undesirable situations seems to remain the same. The problem in my case was 350 freelance writers who were looking for interesting angles, which primarily meant interviewing me, the expedition leader, and my colleague and friend Dr Gerald Wick. However, as the big day approached, we were pretty much left alone, and I was able to take a closer look at my fellow passengers. They were a most interesting bunch. Astronaut Scott Carpenter, along with several members of New York high society, were there. So were secretaries, nurses, and several professional astronomers.

People have responded to solar eclipses in a variety of ways. One king, Louis of Bavaria, was frightened to death by it. A solar eclipse stopped a battle between the Lydians and the Medes, and, as late as 1948, the Korean national elections were postponed, again because of an eclipse. American Indians have described eclipse as "the sun being very sick and going to bed"; and India's Indians have interpreted them as the sun being devoured by evil spirits. Our fellow passengers might have lost some of the fear which beset their early ancestors, but definitely not their reverence and enthusiasm. When I asked several of them how it felt to witness an eclipse, the answers were invariably the same: "There is nothing like it". Like what? Few people seemed to know, or were prepared to say. Yet Leif Robinson, the associate editor of *Sky and Telescope* and a fellow passenger

on board the *Olympia*, probably summed up best what it is about eclipses that forces us to follow them wherever they take us: "Every solar eclipse unfolds in a predictable way, only the details changing. It is the manner in which these subtleties of colour, brightness, and form are blended that gives each eclipse a flavour as distinct as vintage wine". Conrad would have liked that.

It did not take me long to discard my idea that the passengers were on this cruise to have a good time, in addition to watching the eclipse. It was the eclipse and the eclipse alone that they were after, and many, I am sure, would have swum the distance if that were the only transportation possible. Nevertheless, the entrepreneur of the cruise, Phil Siegler, a social scientist from New York, had also insured a full house by offering something called 'Science at Sea'. Courses ranging from astronomy to bird-watching were taught by astronomers, atmospheric scientists, Scott Carpenter and Leif Robinson.

Nor, as it turned out, was our group the only one mixing pleasure with business. Milner Schaefer, the inventor of cloud seeding and a most charming man, was geared to record cosmic rays; and Dr Williams, of Yale University, passed out photocopied notices inviting all interested passengers to share their eclipse-related dreams with him as a part of his research project¹. However, the most important scientist on board was the meteorologist whose primary function was to guide the ship to the most favourable (that is, cloud-free) location. Outfitted in a Scottish tweed jacket and impeccably shiny shoes, he entered into Ahab-like trances which never failed to command our closest attention, and his least utterance about the weather was speedily relayed to the farthest corner of the vessel.

Then it happened. It wasn't what I expected. What surprised and fascinated me was not the sudden darkness which swooped down on us with the grace of a hammer-head entering a South-Sea lagoon, but the grasshopperesque chatter of cameras, all 799 of them. I suddenly realised that I might be the only one who was watching it—the rest were busy recording it for posterity, be it for a lecture demonstration for an undergraduate astronomy class at Harvard, or for a slide show especially prepared for a maiden aunt and her boyfriend in Galesburg, Missouri. What a pity! Thanks to the camera, most people became the lovers of an illusion of reality, rather than of the reality itself.

It is that time again and the faithful are getting ready to watch, on 12 October, the total solar eclipse which, according to reports, will be best viewed off the coast of central America (13° 42'N 123° 07'W). In order to accommodate the large number of applicants, Sitmar Lines is offering two ocean liners, along with such luminaries as Margaret Mead and Isaac Asimov. If you happen to be in the neighbourhood, take a look at it. It is some sight.

As for our experiment², in the finest scientific tradition, the results were rather ambiguous. Even though some of the organisms responded to the eclipse by migrating vertically, some did not. One of the results was totally unexpected: increased bioluminescent activity during totality. One can offer several hypotheses to explain this phenomenon, but I am sure, to the eclipse lover, this was nothing more than the little bugs celebrating the joyous occasion.

¹ For the various scientific projects conducted during solar eclipses, see *Solar Eclipse Bulletin* 1-4 (National Science Foundation, Washington, D.C.; 1973).

² For the results of our experiment, see *Deep-Sea Research* 20, 769-771; 1973.

Two decades of Soviet space

Vera Rich looks at the Soviet space programme, twenty years old this week

ON 4 October 1957 the first artificial satellite, later to be known as *Sputnik 1*, was launched as part of the Soviet contribution to the International Geophysical Year. From the Soviet point of view, the date fell auspiciously close to the 40th anniversary of the October Revolution, and also in the centenary year of the birth of Konstantin Eduardovich Tsiolkovskii, 'the father of cosmonautics'. During the subsequent decade the Soviet space programme notched up an impressive number of 'firsts'—first animal in space, first lunar impact, first circum-lunar probe, first man in space, first (and to date only) woman in space, first interplanetary probe, first automatic docking and separation. The USSR could even claim the first 'space baby', Elena, the daughter of cosmonauts Tereshkova and Nikolaev, whose healthy arrival did much to disperse alarmist fears of the genetic hazards of space flight.

It was not until the late 1960s that the unbroken run of Soviet 'firsts' slowed down. Like the Apollo 8 accident in the USA, the tragic death on 24 April 1967 of Vladimir Komarov, whose re-entry parachutes failed to open properly, put an end to the euphoria. The death a month before of Yuri Gagarin in a flying accident not only deprived the Soviet Union of its greatest living hero, but also appears to have caused a major change in policy. During her post-mission world tour, Tereshkova had told a press conference in Cuba that the Soviet moon team had already been picked and that Gagarin was to lead it. Since his death, no mention has been made of a manned lunar mission in the foreseeable future and, with the success of the US Apollo missions, the Soviet planners have preferred to stress the considerable saving (up to 50%) effected by using unmanned rather than manned lunar probes.

This stress on saving is not simply a face-saving excuse. The actual cost of the Soviet space programme is a closely guarded secret, but from the very beginning there was a strong body of feeling that whatever the cost might be, it was too high. A whole series of black jokes circulated, based on the resemblance of the word for meat (*myaso*) and the archaic name for the moon (*mesyats*), suggesting that the shortages of the one were not unconnected with the rockets to the other. By 1960, *Komsomolskaya Pravda* had to launch a special campaign to quash

such criticism. But public opinion had made its point; the comrade-in-the street was not prepared to accept pie-in-the-sky as a substitute for consumer goods on earth. Gradually it became standard practice to announce all space-missions as being "in the interests of the national economy"—a phrase which seems somewhat hard to justify (except in terms of serendipity) when applied to a Venus or Mars probe.

Terrestrial applications

A considerable proportion of Soviet launches do, of course, have terrestrial applications. The *Meteor* meteorological satellites, and the *Molniya* communications satellites have proved invaluable in the opening up of Siberia, not the least of the advantages of *Molniya* being that it provides the solace of TV for workers on remote construction sites. Visual and infrared photography, from manned and unmanned satellites, has found a wide range of applications from oil-prospecting to fish-spotting. The *Kosmos* series of satellites, initiated in 1962 and now approaching the 1,000 mark, covers a number of such applications. The vagueness of the series title makes it an ideal cloak for any purpose the planners may wish to conceal. In the early days unsuccessful lunar or interplanetary probes were assigned a *Kosmos* number, while *Kosmos 47* was probably an unmanned trial run for the manned *Voskhod 1*, which followed a week later.

Initially one of the basic missions of the *Kosmos* programme was stated to be the monitoring of high-altitude nuclear explosions. *Kosmos 5*, in particular, measured the decay of high-altitude radiation following the American 'Starfish' nuclear test of 9 July 1962. Happily the 1963 atmospheric test ban treaty rendered this part of the programme obsolete. There is little doubt, however, that the *Kosmos* series is not without its military applications; and the term *Kosmos* has also covered various 'occasional' experiments, including automatic docking, and tests on biological specimens.

With the abandonment of any immediate plans for a manned moon mission, the Soviet manned space programme became centred on the concept of orbital space stations—the approach which had been favoured by Tsiolkovskii himself. The first such station, *Salyut 1*, was launched in 1971. Although the success of the mission was overshadowed by the death of the second-shift crew during re-entry, the

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Where it all began: Sputnik 1

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Where the USSR is now: Soyuz 24 about to take off

series continues, and this week's anniversary is marked by the launch of *Salyut 6*, as well as by the publication by the Crimean Astrophysical Observatory of a 'catalogue' of solar radiation of various wavelengths and a detailed spatial distribution of the temperature of solar flares, based on films taken aboard *Salyut 4*.

According to Academician Georgii I. Petrov, the *Salyut* programme is a three-fold one—astrophysical observations, surveying earth resources, and physical experiments associated with weightlessness. Already a number of interesting results have been reported on crystal growth and the cooling of liquid metals in weightlessness; in a recent *Pravda* interview Petrov suggested also that a space station would be a suitable base for producing specimens of ultra-high purity.

He further suggested that such space stations could well provide a base for a long-term moon expedition. He envisaged a lunar-orbital station with a crew aboard; they would send down automatic probes and sampling devices to the surface of the moon, to make observations or bring back specimens as required. "I can see no reason", Petrov remarked, "that would require man to make a long-term stay on the

moon". He then proceeded, not surprisingly, to rule out any possibility of a manned Mars mission, largely on the grounds that the biological effect of long-term weightlessness is still unknown. If weightlessness really presents such a danger, it seems a little remarkable that a Soviet manned 'lunar' mission would preferably be kept in orbit rather than allowed to benefit from the reduced but at least perceptible gravity of the moon.

No further

For the moment, it would appear, Soviet man will venture no further than a circumterrestrial orbit. Not surprisingly, the "most interesting" future experiments which Petrov outlines will all be carried out by unmanned probes—the collection of highly rarefied 'undamaged primary meteorite material', and the recovery of solid material from interplanetary space. Other exciting projects for robot probes include flights over the poles of the sun, and a deep-level descent into the atmosphere of Jupiter. And, said Petrov, the only sure way to solve the age-old problem of whether there is life on Mars is the automatic recovery of a soil specimen from the planet. It is here, incidentally, that Soviet expertise gained during the automatic moon programme will pay off. The specimens of lunar soil recovered by the *Luna* probes were far smaller than those brought back by the Apollo crews, a fact which the US media were not slow to note and which the Soviet publicists played down by stressing the technological prowess behind automatic sampling. For Mars, however, automatic sampling will probably antedate any manned mission by a number of years, while in the case of Venus, automatic sampling would appear essential. Similarly, a roving vehicle of the *Lunokhod* type could easily be redesigned as a *Marsokhod*, with only minor modifications to the Martian environment.

To launch such missions, there are, according to Petrov, "lively discussions" about the possibility of a "reusable" launch craft similar, presumably, to the US Space Shuttle but possessing, if necessary, two returnable launch stages.

International cooperation

One aspect of the space programme was not mentioned by Petrov—international cooperation. That is the responsibility of his namesake, Academician Boris N. Petrov, the head of the *Interkosmos* programme. *Interkosmos* itself is the space research organisation of the Comecon block, launching the astrophysical *Interkosmos* satellites (17 to date) and the *Vertikal* high-altitude rockets. However, the Soviet Union also has programmes of space cooperation with other countries,

Novosii

1967: Kosmos 186 and Kosmos 188 approach for automatic docking

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Novosii

Training in capsule recovery in water: normal Soviet style is over land

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notably France, India, Sweden, and the USA. The type of cooperation varies from country to country.

To date the Franco-Soviet programme has been the broadest based, and has included geodesy, aurora borealis/australis, and interplanetary particle flux experiments, as well as laser ranging to the moon, using French reflectors on *Lunokhods* 1 and 2. A Swedish experiment on ultraviolet solar radiation was carried by *Interkosmos* 16 under an agreement with the Soviet Academy of Sciences, and a new cooperation agreement signed last month will provide for Swedish magnetosphere-plasma experiments to be carried by Soviet high-apogee satellites.

The most significant cooperation agreement of the whole 20 years, how-

ever, was that between the USA and the Soviet Union, which culminated in the Apollo-Soyuz link-up of July 1975. The space age began in an atmosphere of mutual doubt between the superpowers—the Soviet triumphantly, the USA fearfully assessing the propaganda advantage of *Sputnik* 1. It took several years for even so innocuous a matter as the exchange of high-altitude meteorological photographs to become a matter of practical politics. The political and psychological implications of space-flight are too large a subject to be dismissed in a paragraph; nevertheless it may well be that *détente* on the one hand, and the growing concern with conservation and ecology on the other, are themselves as much a product of the space age as an Apollo capsule or a Venus descent craft. □

CANADA

Digesting the food problem

David Spurgeon writes from Ottawa on the impact of a symposium held in August to consider how Canada could best contribute in the future to the alleviation of world food shortages

A SYMPOSIUM on the theme "Canada and World Food" has had the paradoxical effect of bringing to the fore some deep-seated concerns about the state of Canadian agriculture itself. Sponsored by the Royal Society of Canada and the Agricultural Institute of Canada, and featuring some leading figures of Canadian and US agriculture and foreign aid programmes, the meeting also brought into question some of the country's official food aid policies.

Some consensus was reached at the meeting. There seemed to be general agreement that the world food supply was precarious, but that world agriculture was capable of producing enough food to meet demand in the near term. There also appeared to be general agreement with the idea that the world's food security was a political and not an economic or scientific question. Too little emphasis was seen to be placed on agriculture by developing countries.

But the symposium also brought out wide differences of opinion on the effects of food aid and its consequent value to developing nations. On the Canadian position, for example, Gordon A. MacEachern, president of the Agricultural Economics Research Council of Canada, had alarming things to say. He maintained there had been "a serious deterioration in Canada's agriculture-food industry output, trade, productivity performance, and a serious decline in our relative contribution to world food, compared to earlier periods and the performance of other net food exporters, most notably the United States".

Though others pointed out that Canada currently is the world's second largest supplier of food aid, MacEachern said there seemed

little benefit in addressing the relatively attractive world food demand prospects, or the economic potential for Canada expanding its agriculture-food capacity to play a greater role in world food supplies, when its cost-price structure says it can't compete. Pervasive voices from almost all segments of Canada's agriculture and food industries are echoing these sentiments, as are senior agricultural officials in Canada, suggesting that what is presently at stake is the very survival of our agriculture-food industries.

Canada continues to enjoy a favourable trade balance in food, as it always has. But over the past 20 years the country's food and feed exports have not kept pace with the growth of either the Canadian or the world economy or the growth in world food and feed trade. In the past five years, Canada has maintained a net export position for only a few commodities such as grain and oilseeds, and in these output and export performance have remained more or less static.

One reason for this, said MacEachern, is the steady growth in Canada's food imports, two-thirds of which are similar to or the same as foods that can be grown in Canada. Other reasons included the artificial support of the Canadian dollar by the federal government, the high level of wage rates in the industry in Canada, and an effective level of protection of 20-40% for manufacturing industry, which further raised agricultural costs.

Len Shebeski, dean of the University of Manitoba's faculty of agriculture, quoted a pessimistic comment made in June by Dr Fred Bentley of the University of Alberta's soil science department, who is chairman of the board of

trustees of the International Centre for Research in the Semi-arid Tropics (ICRISAT) in India. Without a massive and well-coordinated effort to meet the needs of agriculture, he had said, Canada could become a food deficit nation before the end of this century. Shebeski disputed this. He believed that Canada's agricultural potential was even greater than the 65 and even 100% increase over present production others had suggested. He based his prediction on the possibility of doubling the land area devoted to agriculture and a 60% increase in yield per acre through improved management. Agricultural lands in Canada had the potential—with present technology—for "more than three times present production of field crops and more than ten times the present ruminant livestock production". And these estimates could be revised upwards "if adequate moisture supplies in the more arid regions of western Canada could be assured."

Shebeski wondered, however, whether Canada ought to reach such production figures. "Because Canada, territorially, is the second largest country in the world, there has developed a popular belief—often voiced—that it is the bread basket of the world," he said.

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MacEachern acknowledged that

Indian wheat harvest: but how to assure supplies?

Those of us involved in agriculture know that this is a myth. An examination of a world map depicting the agriculture lands of the world shows that Canada's component of the world's present 3.6 billion acres of crop land is approximately 5% . . . Canada's strategic position in the current world food situation does not arise from a high tonnage of food outputs, but rather from the large proportion of her total production available for off-shore consumption.

This production, he intimated, should not be relied upon freely by developing countries to fill their food shortages. The costs of claiming the 20 million hectares of potentially arable land of low fertility would be enormous. Crops produced would be modest in quantity and marginal or submarginal in profitability. The food produced would be thousands of miles from where it would be needed.

As an alternative, Shebeski proposed that the Canadian government, through the International Development Research Centre (IDRC) or the Canadian International Development Agency, make available to developing countries the money it would cost to bring large areas of potential Canadian land into food production. This money could then be used to bring into production land areas with the agricultural potential of the Indus-Ganges-Brahmaputra plain of North India, or parts of Brazil, where experiments have shown production possibilities of more than 70 bushels per acre. He had struck on a note that was echoed in much of the conference: investments in food production to meet shortages in developing countries should be made in those countries rather than in developed ones.

David Hopper, president of the IDRC, said food assistance should not be denied in cases of genuine emergency or hardship. But imports had in some cases in the past held domestic farm prices below levels that would have prevailed in the recipient countries had the imports not been available, and this had destroyed the incentives to generate local increases in food production. Where the food aid was essentially free, its sale within the country by the recipient government had directly augmented national treasury resources, reducing the need to tax more heavily or to encourage larger savings for domestic investment programmes. That blunted incentives for local development, and removed any urgency to look further into measures to promote the more rapid development of indigenous farming.

Fred Bentley proposed that, as a minimum, every food-deficient less-developed country should be required to increase the budget for agriculture by an amount at least equal to the value of food aid received, without any diminution in the percentage of the

national budget already being allocated to the agricultural sector. Alternatively, recipients could add the funds to their population control programmes. But T. K. Warley, of the School of Agricultural Economics and Extension Education at the University of Guelph, Ontario, defended food aid, saying it had served many purposes beyond providing increased food supplies to hungry people in poor countries, including the general transfer of resources to developing countries, support of the balance of payments and thus maintenance of high rates of economic growth, and promotion of political stability and allegiances. Food aid, he said, should be used to accelerate agricultural and rural development, alleviate hunger in specific target groups, and help build minimum stocks of basic foodstuffs.

Frank Shefrin, director of the Canada Department of Agriculture's international liaison service, summed up the magnitude of the world problem. He said that it was not enough to increase the production of food. There had to be increases in food production in the poorer countries and by the small farmers, so that imports of food would not exceed their means of paying. The food had to be produced where it was needed and get to those who need it. Just to stay at the present per capita food production, developing countries could require an annual investment in agriculture of about \$20 billion, of which 60-70% would have to come from the countries themselves unless there was a substantial increase in capital assistance.

Findings of the latest report of the International Food Policy Research Institute (IFPRI) in Washington were also revealed. Projecting food shortfalls of some 82 developing countries (containing roughly half the earth's population) by comparing prospective food production if performance continues in the next 15 years as it has in the past 15, IFPRI has come up with the following forecasts:

- A total food grain deficit among those countries with less than \$300 GNP per capita in 1973 of 70-85 million tons by 1990 (12 million tons in 1975).
- A need for some 35 million metric tons over and above the projected production in 1990 just to provide for population increases in these countries (maintaining per capita consumption at 1975 levels).
- Potential shortfalls by 1990 among all developing countries included in the study of 120-145 million tons, a figure which approaches the total volume of world grain trade in recent years.
- A potential shortfall of 21-25 million tons by 1990 in India, which has almost half the people in low-income

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Onloading in Canada

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Offloading in India

food deficit countries. To meet this, an increase in production of 3½% a year would be required, compared to the historical 2½%.

- Deficits of 6-8 million tons each for Bangladesh and Indonesia. For Bangladesh, production would need to increase almost 4½% a year compared to a historical rate of 1½%. For Indonesia, the rate should be 4½% instead of the historical 3%.
- A deficit of 18-21 million tons facing Nigeria, which would require 5-5½% increase a year (historic rate ½%).
- An increase to 3½-4% required in Sahel countries to avoid a shortage of 3½ million tons (historic rate -½%).
- To meet nutritional needs, most low-income food-deficit countries need an additional ½-1% growth rate over and above those necessary to meet market demand. □

USA

Department store

David Davies reports from Washington on the new US Department of Energy

THE Department of Energy, the twelfth Cabinet agency of the United States, comes into being on 1 October. Proposed by President Carter on 1 March of this year, the department will be headed by Secretary James Schlesinger. It will have almost 20,000 employees and a first-year budget of \$10,400 million. An amalgam of several agencies, most notably the Energy Research and Development Administration (ERDA) and the Federal Energy Agency, the new department's structure is a little unusual in that it is divided along functional lines rather than technological lines. Thus technologies will move through different parts of the department as they progress from basic research through development and into commercial implementation.

Responsibility for basic research will lie, in large measure, in the Office of Energy Research, which will manage basic science programmes both within the department's own laboratories and in the universities. The Director of this office will be Professor John Deutch, a physical chemist from the Massachusetts Institute of Technology. Deutch, 39, is no stranger to the Washington scene. In the 1960s he worked as a systems analyst in the Department of Defense, and consulted for the Bureau of the Budget. He has been a part-time member of the Defense Science Board, most recently as its Vice-Chairman. But he declares, indeed promises, that in several years time he will be back doing chemistry.

In the meantime he will play four major roles. He will provide Schlesinger with technical advice on basic science issues. He will co-ordinate

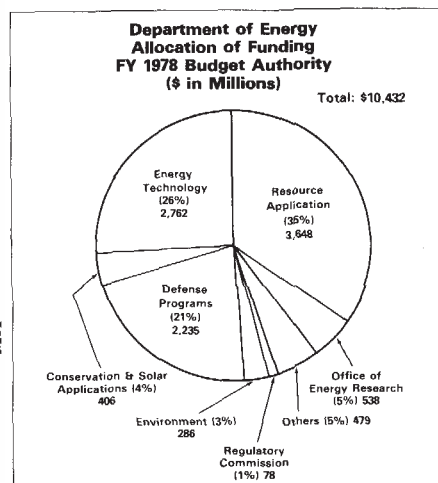
all the department's basic research, some of which will obviously be done outside the immediate orbit of his office. He will be responsible for the well-being of all the department's 144 laboratories (excluding the weapons laboratories). And he will be responsible for the financial support of basic research.

A very substantial fraction of the basic research money at present goes to high energy and nuclear physics, and clearly the future of this research is not too closely tied to immediate questions of energy policy. But in fiscal year 1977, \$125 million was spent in basic energy research, divided roughly 7:3 between governmental laboratory and university recipients. There is little doubt that this sum will rise sharply in the next few years; already federal financial obligations to energy R&D as a whole in fiscal year 1978 are seen as rising by at least 10% in real terms over the 1977 figure.

The department has an Under Secretary and six Assistant Secretaries on the technical side. Subject to confirmation, the Under Secretary, who will oversee all the programmes but have a special brief for energy conservation, is Dale Myers, at present a Vice-President of Rockwell International. Apart from basic research the Assistant Secretaries also cover energy technology; resource applications, with responsibility for the commercial introduction of new technology; conservation and solar applications, a contact point for individual inventors and small businesses; environment, with responsibility for ensuring that programmes comply with environmental health and safety regulations and with the authority to conduct related research; and defence programmes, directing nuclear weapons research and investigations into laser fusion. □



Research director: John Deutch



DNA bill delay

RECENT moves on Capitol Hill have created further confusion in the already contorted story of the various pieces of pending legislation to control recombinant DNA research in the United States. In the House of Representatives, the bill drafted by Rep. Paul Rogers' Health subcommittee was scheduled for consideration by the Commerce Committee last week and was not expected to run into much trouble. Commerce chairman Harley Staggers postponed consideration of the bill, however, and suggested that important amendments might be required. Further amendments have been proposed by other committee members. The chances that the bill will be approved by the committee and reach the floor of the House before the end of the legislative session have now diminished almost to zero.

In the Senate the situation is more confusing. The Kennedy bill which was already running into trouble with Senators concerned about its bureaucratic provisions has been removed from the legislative calendar. Senator Kennedy now proposes to set up a Study Commission composed of interested members of lay and scientific organisations to consider the best form of legislation. As a stopgap, a simple measure to give the NIH guidelines the force of law would be enacted. This would apparently contain no enforcement provisions but would apply to industry as well as government-financed research. The position of the bill recently drafted by Senator Gaylord Nelson, which is similar in many respects to the House bill (see *Nature*, 1 September) is not clear, although it does not now appear to be gathering sufficient support for passage this session.

In addition Senator Adlai Stevenson III, as chairman of the Subcommittee on Science, Technology and Space of the Senate Commerce Committee, has announced hearings on the effect of the proposed legislation; at the same time he expressed strong misgivings about the pending bills and their effect on basic research and freedom of scientific enquiry.

All these moves owe something to the lobbying efforts of some scientists and scientific societies, together with statements on the relative risks and benefits of recombinant DNA research from a number of scientific meetings over the summer. More battles are in store before legislation finally emerges.

Sandy Grimwade

IN BRIEF

UK sweetener review

The UK Food Additives and Contaminants Committee is to conduct a full review of the regulations controlling the use of artificial sweeteners in food. The committee, which falls under the aegis of four governmental departments, will thus extend its present studies of saccharin and of Aspartame; it has published two reports on cyclamates in the past.

Waste now, power later

A three-member panel of the White House Council of Environmental Quality has, according to the *New York Times*, reported that an expansion of nuclear power in the United States should be made conditional upon a demonstrably safe method of containing nuclear waste. The verdict is seen less as a counter to President

Carter's energy plan than as an attempt to encourage work on waste disposal.

IAEA reprocessing view

Though it was generally accepted that the number of nuclear fuel reprocessing plants should be kept to a minimum, to prohibit them would probably lead to a result opposite of that intended, according to Dr Sigvard Eklund, the Director General of the International Atomic Energy Agency (IAEA). He was speaking at the IAEA General Conference's 21st Regular Session in Vienna last week. Dr Eklund, who was appointed to his fifth consecutive term as director general, was also critical of those who wanted to eliminate the nuclear option.

Accelerator approval

Approval has come for the construction of Brookhaven National Laboratory's 200 GeV superconducting

intersecting storage ring accelerator Isabelle. A joint US House-Senate committee last week authorised expenditures totalling nearly \$200 million. The device will enable protons to be fired at each other with centre of mass energies well in excess of those available elsewhere, providing detail on how and if the weak and electromagnetic interactions are unified.

HSC report

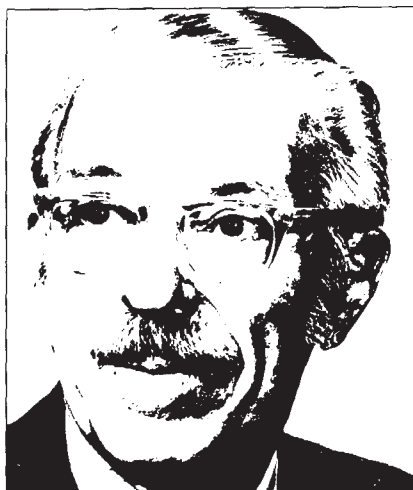
A broad-ranging 51-page report from the UK Health and Safety Commission, published this week, details the work of the Commission and its Executive between their establishment in October 1974 and January 1975 respectively and March last year. The Commission emphasises that legislation can play only a part in achieving the objectives of the Health and Safety at Work Act, and indicates in its discussion of future strategy what more can be done.

THE field of molecular evolution deals largely with comparisons of the sequences of 'informational macromolecules'—DNA, RNA and proteins. The first sequences that were available were of proteins: cytochromes *c* and haemoglobins, from different animals. The story is now familiar: the number and location of differences in corresponding amino acids were roughly proportional to the taxonomic separation of two species. For example, human and rhesus monkey haemoglobins differ in 12 locations; human and horse haemoglobins in 42; rhesus monkey and horse in 43. The differences between human and horse are not all in the same places, or of the same kind, as the differences between monkey and horse, thus showing that the three species have followed separate evolutionary pathways. These findings expanded and accumulated for various families of proteins, and were extended into comparisons between insects, green plants, yeasts and moulds. Various authors constructed large 'phylogenetic trees' based on such findings. It became evident that a 'molecular evolutionary clock', ticking away through the aeons, left its imprint in terms of divergence between sequences. The clock did not always run at the same rate, but, in the main, the divergences roughly corresponded to the passage of time as judged by other criteria, such as the fossil record.

But wait! The differences between proteins were merely the expression of changes in the base sequences of

'codes'. Quite often, two to four DNA molecules as reflected by the genetic code. And the genetic code, or amino acid code, is ambiguous in certain respects, because 18 of the 20 amino acids have from two to six

Molecular evolution



THOMAS H. JUKES

codes for the same amino acid differ only in the third base of the nucleic acid codon that specifies the amino acid. So a change of one amino acid, as measured in a protein, could reflect more than one change in a gene. Various 'educated guesses' were proposed to calculate the actual numbers of such unknown changes.

The discovery of procedures for

rapidly determining the sequences of long segments of DNA molecules is going to revise all this. One such method came from Sanger's group at Cambridge, and, more recently, the field has been galvanised by a chemical procedure devised by Maxam and Gilbert at Harvard. Such sequences can be matched against a protein sequence, even a partial one, and the exact code for each amino acid can be 'read off'. Furthermore, comparisons of DNA sequences obtained from homologous regions in related organisms tell precisely which nucleotides have undergone change during the period of evolutionary separation. There are preliminary indications that the synonymous 'third bases' of codons may undergo such changes more frequently than the first two bases, which specify amino acids.

New techniques, such as automated Edman degradation, are improving the accuracy of measuring amino acid sequences in proteins. A sequence between the 23rd and 30th residues in cytochrome *c* of *Neurospora crassa*, previously listed as -Gly-Glu-Gly-Gly-Asn-Leu-Thr-Gln- was found to be -Thr-Leu-Glu-Glu-Gly-Gly-Gly-Asn-. This was a bit of a shocker, and it called for some revision of evolutionary comparisons, but it is probably an extreme case. Other re-evaluations, and an expansion of ideas on evolution, will stem from forthcoming studies of DNA molecules. These studies should shed new light on the question of 'neutral' or 'near-neutral' evolutionary changes that occur in genes.

news and views

Molecular basis of plant tumour induction

from James A. Lippincott

THE long sought tumour-inducing principle (TIP) produced by the bacterium *Agrobacterium tumefaciens* and responsible for the transformation of normal plant cells to tumour cells (crown gall) seems now to be largely identified. This bacterium is known to cause transformation in a wide variety of plants when it infects wounded areas. Unlike cells of the nitrogen-fixing nodules induced by rhizobia, however, these tumour cells do not contain bacteria, and the bacterium needs to be present for only hours to a few days to induce tumours which will continue to proliferate in their absence. This growth autonomy is maintained in tissue culture where the tumours do not require hormones for growth, as do most normal tissues. The tumours also continue their characteristic growth when the bacteria-free cultures are grafted on normal plants. This evidence suggested that a TIP was produced by the bacterium and could alter the growth and developmental potential of plant cells in an essentially permanent fashion.

A major break in the problem came with the demonstration that tumorigenic strains of *Agrobacterium* carried a large ($>110 \times 10^6$ daltons) circular extrachromosomal DNA element or plasmid, whereas several non-tumorigenic strains did not (Zaenen *et al. J. molec. Biol.* **86**, 109; 1974). One strain of *Agrobacterium* readily loses virulence when grown at 37 °C (Hamilton & Fall *Experientia* **27**, 229; 1971) and this was shown to be accompanied by plasmid loss (Van Larebeke *et al. Nature* **255**, 742; 1974; Watson *et al. J. Bact.* **123**, 255; 1975). Genetically marked non-virulent strains of *Agrobacterium* had been found by Kerr (*Nature* **223**, 1175; 1969; *Physiol. Plant Pathol.* **1**, 241; 1971) to

acquire virulence after several weeks when co-inoculated on plant hosts along with virulent bacteria. This gain of virulence was also shown to depend on the plasmid and results from the transfer of a plasmid *in planta* from the virulent to the non-virulent strain (Watson *et al. op. cit.*, Van Larebeke *et al. Nature* **255**, 742; 1975). The necessity of the plasmid for tumorigenicity was thus firmly established. More conventional methods of plasmid transfer have since been developed and results from these tests fully support the initial findings (Levin *et al. J. Bact.* **127**, 1331; 1976; Chilton *et al. Genetics* **83**, 609; 1976; Kerr *et al. Nature* **265**, 560; 1977; Genetello *et al. Nature* **265**, 561; 1977).

Direct evidence that the *Agrobacterium* plasmid is the TIP or at least a major essential element of this concept has now been obtained. Chilton *et al. (Cell* **11**, 263; 1977) have isolated DNA from normal and crown gall tumour tissue cultures and hybridised it with different restriction nuclease fragments of the *Agrobacterium* plasmid. Most plasmid fragments do not hybridise with either normal or tumour DNA. Two plasmid pieces, however, hybridise with tumour DNA but not normal DNA, indicating that the tumour cells have acquired plasmid DNA sequences as a consequence of the transformation process. About 20 copies of a $3.7\text{--}6 \times 10^6$ dalton plasmid segment are estimated to be present in each tumour cell, sufficient information to code for about 10 different proteins.

Further support for these results, obtained by hybridising RNA isolated from tumour cells and normal cells with nuclease fragments of the plasmid, is reported by Drummond *et al.* in this issue of *Nature* (page 535). RNA transcripts of one plasmid segment are found in the tumours but not in normal tissues and this segment is the same as one detected by DNA-DNA

hybridisation experiments. This transcription of the transposed plasmid segment by the plant tumour cells suggests that it codes for one or more proteins which could have functional significance in inducing and/or maintaining the tumour syndrome. A major role of the bacterial plasmid, therefore, is to contribute a segment of new genetic information to the plant cell, and the translation of these plasmid genes in the plant, as suggested by the RNA transcripts, may be essential to the process. In many respects, therefore, the crown gall transformation process seems to parallel the events which occur in the induction of animal tumours by viruses.

Several traits in addition to that essential for virulence have been localised on the *Agrobacterium* plasmid. The ability of the bacterium to utilise either octopine or nopaline, unique N^α substituted derivatives of arginine, was shown to be highly correlated with virulence (Lippincott *et al. J. Bact.* **116**, 378; 1973) and loss or gain of the plasmid is accompanied by corresponding changes in utilisation of these compounds (Watson *et al. op. cit.*; Van Larebeke *et al. op. cit.*). Typically, the tumours synthesise one or the other of these arginine derivatives and the derivative synthesised is the same as can be utilised by the strain which induced the tumour (Petit *et al. Physiol. Veg.* **8**, 205; 1970; Bomhoff *et al. Molec. gen. Genet.* **145**, 177; 1976). A second plasmid gene is responsible for the ability to induce tumour synthesis of these compounds (Klapwijk *et al. J. gen. Microbiol.* **96**, 155; 1976; Montoya *et al. J. Bact.* **129**, 101; 1977).

This remarkable ability of *agrobacteria* to induce plants to synthesise compounds which the bacteria can utilise specifically as both a carbon and nitrogen source may well provide the *raison d'être* of this pathogen-host relationship. The finding that plasmid transfer

is greatly promoted *in vitro* by octopine (Kerr *et al. op. cit.*; Genetello *et al. op. cit.*) suggests that these compounds function similarly in the bacterium-tumour complex, promoting the plasmid exchange observed here. The tumours may thus provide both the stimulus and the nutritional requirement to accomplish gene transfer in the agrobacteria. It is also proposed that the enzymes responsible for octopine or nopaline synthesis in these tumours might be coded by bacterial genes (Petit *et al. op. cit.*) and recent plasmid results suggest that one of the plasmid genes which is transcribed in the tumour could be involved. No agreement exists, however, as to whether these arginine derivatives are normal plant products or are present only in these tumours.

Exclusion of *Agrobacterium* phage API (Van Larebeke *et al. op. cit.*) and, in some nopaline-utilising strains, sensitivity to a unique bacteriocin of *Agrobacterium* strain K84 (agrocin 84, Roberts *et al. Nature* **265**, 379; 1977) is determined by the plasmid (Engler *et al. Molec. gen. Genet.* **138**, 345; 1975; Watson *et al. op. cit.*). Selection for resistance to agrocin 84 frequently results in plasmid loss or a deletion of portions of the plasmid essential for virulence. This has found practical application through use of strain K84 to control *Agrobacterium* infections in horticultural conditions (New & Kerr *J. appl. Bact.* **35**, 279; 1972). The plasmid also carries a gene which determines the ability of the bacterium to adhere to infection sites, an essential step in tumorigenesis (Whatley, dissertation, Northwestern Univ; 1977).

At least two plasmid genes seem to be functional in the transformation process in addition to adherence, because certain non-virulent strains which also carry a plasmid can increase tumour initiation when inoculated with virulent bacteria in conditions where bacterium-to-bacterium plasmid transfer is not observed (Lippincott *et al. J. Bact.* in the press). Indirect evidence suggests that the virulent bacteria produce or induce a diffusible product other than the intact plasmid which permits tumour initiation by the non-virulent strain. Obviously, much has to be learned about the nature of the genes localised on that part of the plasmid which is replicated in the tumours, as well as the genes and functioning of the much greater part of the plasmid which is excluded. The need for such a large plasmid is in itself an enigma. The possibility that *Agrobacterium* chromosomal genes are also directly involved in tumorigenesis is neither proven nor disproven.

Tumorigenic agrobacteria may now be counted the first cellular organisms demonstrated to accomplish in nature

the genetic engineering feat of transfer and stable incorporation of foreign DNA into a eukaryotic cell. These newer results also provide the first convincing indication that DNA from a prokaryotic cell may contain the appropriate base sequences to permit replication, transcription and possibly translation by the normal cellular machinery of eukaryotic cells. While important details of this transforma-

tion have yet to be established, the exciting prospects of genetic engineering for the production of beneficial changes in crop plants assures that they will be forthcoming. The transfer of nitrogen-fixing genes of *Rhizobium* to the closely related agrobacteria and subsequently to various plant hosts is but one example of the possibilities which are already receiving attention (*Science* **196**, 640; 1977). □

Are galactic γ rays point sources or diffuse emission?

from J. J. Quenby

A WEALTH of positive results from the SAS 2 and COS B satellites has firmly established the science of γ -ray astronomy, although as recently as 1970 the usefulness of making comprehensive searches for these high energy cosmic photons was actively debated. However, while the information acquired is clearly significant, what is still not clear is exactly which branch of astrophysics is being served by the measurements. A letter by Hermesen *et al.* in this issue of *Nature* (page 494) illustrates the difficulties involved.

γ -Ray emission is expected from the galactic disk by virtue of the interaction of cosmic rays with the residual matter in the interstellar medium. Although these charged, relativistic, cosmic ray particles may fill an extended galactic halo region (see *Nature* **268**, 401; 1977), it is only in the galactic disk that sufficient hydrogen atoms, at a density of about 1 cm^{-3} , are encountered for detectable γ -ray emission to occur. The π^0 mesons arising from the cosmic ray interactions decay into two γ rays producing a broad spectrum of photons. Any variation in the galactic longitude distribution of the resulting emission from the Milky Way should yield information on the spatial distribution of cosmic rays, provided the matter density is well established from other astronomical evidence.

The surprise arising from the earliest detection of γ rays from near the galactic centre by Kraushaar *et al.* (*Astrophys. J.* **177**, 341; 1972) on board OSO-111 was the unexpectedly high intensity, implying that cosmic rays are much more numerous at the centre than far out in the disk, near the Sun.

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Maybe there are more cosmic-ray sources at the centre of the galaxy, but particle diffusion along the spiral arm magnetic fields needs to be well constrained to maintain this apparent gradient in number density. The SAS 2 γ -ray detector provided the first comprehensive survey of the galactic plane emission (Fichtel *et al. Astrophys. J.* **198**, 163; 1975) and the results show a reasonable correlation of γ -ray intensity with matter density in the spiral arms lying between us and the galactic centre. Furthermore, some of the discrepancy between the observed flux and that expected if cosmic rays were uniformly distributed in the Galaxy has been removed by including in the matter density the amount of molecular as opposed to atomic hydrogen, as estimated in an analysis performed by Scoville and Solomon (*Astrophys. J.* **187**, L67; 1974). However, an increase of a factor of two in the cosmic ray intensity is still needed in the inner spiral arm regions (Stecker *Phys. Rev. Lett.* **35**, 188; 1975). Moreover, at the Cosmic Ray Conference this August, several workers were concerned whether or not cosmic rays could actually easily penetrate the expected high magnetic fields within the dense clouds where molecular hydrogen is important (Cesarsky *et al.* and Strong *et al. Plovdiv C.R. Conf.* **1**, 61, 55; 1977).

The ESA satellite COS B is devoted entirely to a γ -ray detector constructed by six West European groups and data from this instrument have now provided more detail on parts of the galactic disk longitude distribution of emission. A particular feature of the results is the number of 'point' sources revealed in an incomplete survey with a 2.5° angular resolution. In 160° of longitude, Hermesen *et al.* find 13 sources,

10 of which are COS B discoveries. Two sources, the Crab and Vela, are known pulsars in supernovae remnants, one is possibly the strong X-ray source Cyg X-3, but the other 10 have either a weak or no association with soft X-ray sources or radio pulsars. They may be point-like or emission regions up to 2° wide. One may be associated with a source of hard X rays seen by Ariel 5 (Coe *et al.* *IAU Circ.* No. 3003, 1976). Hermesen and colleagues stress that the γ -ray sources contribute significantly to the overall γ -ray luminosity of the Galaxy. Indeed, as pointed out by Strong *et al.* (12th *ESLAB Symposium, Frascati*, 1977), removal of point sources leaves very little evidence for a diffuse galactic emission from and beyond the Perseus arm, just outside 10 kpc from the galactic centre. It is tempting to conclude from the much increased fine structure seen in the COS B γ -ray intensity distribution that point source emission dominates and that little information on galactic cosmic ray motion can be gained by study of the disk photon emission.

Attacking the problem from the other end of the spectrum, the

Imperial College group have suggested that at least 8 (maybe >16) galactic hard X-ray sources may plausibly be extrapolated into the γ -ray region to account for a significant amount of the local γ -ray intensity (Plovdiv *C.R. Conf.* 1, 152; 1977). However, conventional accretion models for X-ray sources involving essentially thermal heating processes have difficulty in explaining γ -ray emission and a young pulsar model requiring electromagnetic acceleration would seem to be necessary, as invoked by Lamb *et al.* (*Astrophys. J.* 212, L63; 1977) to explain possible γ -ray production in Cyg X-3.

Until the contribution of point sources to the galactic disk emission is resolved, γ -ray astronomers cannot know whether they are chiefly contributing to knowledge of cosmic ray motion in the Galaxy or studying an important class of high energy photon sources involving non-thermal particle acceleration. Better angular resolution and sensitivity throughout the hard X-ray and γ -ray detector range is required to resolve this particular problem. $\square$

Polarisation in quasars

from F. Graham Smith

THE famous quasar 3C 273, which was the first to be shown to be both a radio source and an optical object with large red-shift, has been studied by every conceivable technique at radio, infrared, optical and X-ray wavelengths. It has spatial structure, a complex spectrum and polarisation, all of which vary with time scales between days and years. The physical processes behind these variations are practically unknown, and observers must sometimes despair of the possibility of making the key observation which will lead to an unravelling of the complex knot of behaviour. The paper from the Crimean Observatory (in this issue of *Nature*, page 493) puts a little order into the great variety of behaviour by showing that some of the radio and optical variations are related. The work has involved measurements of polarisation, both optical and radio, which have been difficult to make and which were for some time in doubt.

Variations in radio flux from 3C 273 were well known in 1966. They are most marked at short wavelengths: at 40 cm not much happens, but at 2 cm the flux density varies by a large factor. The spectra of several quasars

show these variations, which indicate an activity close to the core of the quasar, giving a rapid rise in radio emission first at short wavelengths and later, with less intensity, at longer wavelengths. Aller and Haddock at Michigan found that these variations were accompanied by changes in radio polarisation.

Optical polarisation was naturally searched for at this time, and indeed there was already a positive result by W. Liller in 1963, who reported verbally to the American Astronomical Society. Appenzeller concluded in 1968 that there was 0.26% linear polarisation, probably of interstellar origin. Results from the 2.6 m Crimean telescope then suggested the existence of circular polarisation, but at such a low level that the techniques were questionable. The observations were made at the coudé focus after several reflections, which easily create spurious polarisation. In 1972 these observations were restarted at the Cassegrain focus, with a more symmetrical optical system and checks with stars with well established circular polarisation showed a measuring accuracy of 0.01%. Both radio and optical observations of polarisation are achieving truly remarkable sensitivity.

3C 273 has an optical jet, a detached radio source (A) and a central source in which the radio object 3C 273 B has at least three components with milli-arc s diameters. Recent long baseline interferometer measurements (Legg *et al.* *Astrophys. J.* 211, 21; 1977) show that the radio variability is concentrated in one of these three components, on the NE (anti-jet) side of the nucleus. Assuming that the radio emission is synchrotron, the magnetic field in this component appears to be about 10^{-2} gauss (10^{-6} T). This field is very low in comparison with the stellar surface fields usually responsible for circular polarisation and it seems unlikely that this variable radio source can be the origin of circularly polarised optical emission. This must instead be related to the nucleus where much stronger magnetic fields might be encountered. Presumably the linear polarisation which is now observed to vary also originates in the nucleus.

The interesting aspects of the observations now reported are the rapidity of the variations both at radio and optical wavelengths, and the connection they seem to give between the NE radio component and the nucleus. If the results can be confirmed they should give a more coherent picture of the way in which the nucleus is pumping energy into the outlying sources, which is a crucial question in the origin of the extended radio sources frequently associated with quasars and radio galaxies. $\square$

Electrophysiology of the hypothalamus

from J. L. Barker

A symposium on the Electrophysiology of the Hypothalamus was held at Hemingford Grey House in Cambridge on 24-26 July in conjunction with the 27th International Physiological Congress in Paris. It was organised by R. Dyer (ARC Cambridge) and R. Dyball (ARC Cambridge).

THE hypothalamus is associated with many bodily functions including autonomic reflexes, feeding and sexual behaviour, neuroendocrine control of anterior pituitary activity, thermoregulation and probably, metamor-

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phosis and hibernation. The symposium produced illuminating discussion on the electrophysiological basis of these involuntary activities crucial to the survival of the animal.

The initial aim of those in the field has been to correlate specific circuits with specific functions. However, unlike the cerebellum, cortex and hippocampus, where considerable circuitry has been delineated but where function remains unclear, the hypothalamus is replete with many short-range neurones and complex connectivity, making simple correlation of excitability with specific functions exceedingly difficult. In spite of this many of the contributors to the symposium attempted this correlation.

Those investigations focused on the magnocellular nuclei in the hypothalamus have achieved some measure of success, owing to the apparent homogeneity of magnocellular neurones and their dominant projection to the posterior part of the pituitary. Two populations of neurosecretory cells in these nuclei synthesise, transport and secrete vasopressin and oxytocin into the peripheral circulation to regulate plasma osmolarity and various types of smooth muscle contractility. The long projections of the cells have been used to locate the parent cell bodies in the nuclei, since electrical stimulation of the terminal causes 'antidromic' activation of the cell bodies, thus indicating whether the neurone recorded from projects to the posterior pituitary.

Significant contributions to the physiology of these neurosecretory cells have been derived from experiments using natural stimuli. These studies show that one population of neurones is induced to fire in a characteristic 'phasic' manner following circulatory compromise while another group is activated by suckling pups (J. D. Vincent and D. Poulain, University of Bordeaux; Dyer, Dyball and J. B. Wakerley, ARC Cambridge; D. W. Lincoln, University of Bristol; J. J. Dreifuss, Ecole de Médecin, Geneva; H. Yamashita, State University of New York, Brooklyn). The former are assumed to be vasopressin-secreting neurones, while the latter are considered to be oxytocin-secreting cells, because the two different stimuli have been shown to release in a specific and independent manner each of the two neurohormones. Thus, two populations of neurones in the magnocellular nuclei have been tentatively identified by their electrophysiological responses to natural stimuli. The presumed vasopressin cells seem to generate phasic activity independent of each other, while the presumed oxytocin neurones fire high-frequency bursts in a synchronised manner. Not yet established is whether the phasic firing pattern is

a latent, endogenous property of these cells which is activated with appropriate stimuli, as happens in a variety of invertebrate neurosecretory cells, or whether the pattern is synaptically derived. Likewise, the synchronised burst of oxytocin neurones may represent electrical coupling among the elements, as occurs in some invertebrate neurosecretory cells, or it may reflect common synaptic input. Definite relationships between hormone release and electrical activity were demonstrated by Dyball, Lincoln and K. Boer (Netherlands Institute for Brain Research, Amsterdam), all of whom showed that clustering of spike activity into phases or bursts produces considerably more neurohormone output than random, unclustered spike activity. Some clue to membrane mechanisms underlying this facilitated, pulsed release is suggested by studies on invertebrate neurosecretory cells which show that the conductance responsible for spike repolarisation accommodates during bursts (J. L. Barker and T. Smith, National Institutes of Health, Bethesda), thus progressively prolonging individual spikes in a burst and allowing facilitated entry of Ca^{2+} and release of neurotransmitter or neurohormone. Pulses of oxytocin are apparently necessary for lactation, since a constant, low level of hormone is ineffective.

Although considerable effort has been expended on the parvicellular elements in the hypothalamus, most notably by L. P. Renaud (McGill University, Montreal), R. L. Moss (University of Texas, Dallas), B. Dufy (University of Bordeaux), M. C. Harris (University of Birmingham) and K. Yagi (Jichi Medical School, Tochigi-Ken), definite conclusions regarding the roles of these cells in hypothalamic function have yet to be made. This impasse derives mainly from the interconnectivity of the cells, as well as a lack of systematic identification of recorded cells by combined marker stains and immunohistofluorescence techniques. The complexity and lack of clear organisation among these hypothalamic elements has caused immense difficulties in specifying the circuits and electrophysiological bases of thermoregulation, sex, autonomic and nutritive behaviour. Some progress has been made by E. T. Rolls (University of Oxford) who has demonstrated hypothalamic units specifically responding to visual stimuli conveying information about food. Mapping of those units conveying information about central and peripheral temperature is being done by those interested in thermoregulation, including R. F. Hellon (National Institute for Medical Research, London), D. Ford (St Bartholomew's Hospital, London),

J. A. Boulant (Ohio State University, Columbus), and J. T. Stitt (Yale University, New Haven). Another line of research, using the push-pull cannula method to apply to, and sample from the hypothalamus various neuroactive substances, has revealed some of the substances released during specific behaviour (R. D. Myers, Purdue University, West Lafayette).

One may conclude from this symposium that the understanding of the cellular basis of assorted hypothalamic functions is fragmentary at best, and that although definite progress has been made, far more research, making use of natural stimuli and multidisciplinary approaches, is required to begin to unravel what is certainly an immensely complex area of the nervous system. □

Identifying carcinogens

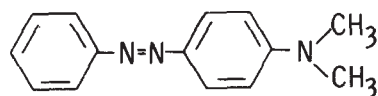
from Alastair Hay

An Ecotoxicology Workshop was held at the University of Surrey from 11 July to 5 August under the auspices of the Eco-Sciences Panel of NATO. The third week of the workshop was taken up by an International Symposium on Industrial Toxicology—the seventh such meeting held at the University of Surrey.

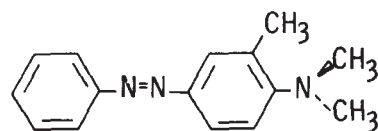
SOME of the problems in identifying possible carcinogens were exemplified during the workshop by work presented on the azo dye butter yellow (I) and the 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) released in the Séveso accident. Butter yellow is a known liver carcinogen whereas TCDD is as yet only under suspicion (see *Nature* **258**, 2; 1975) but reports of the behaviour of these chemicals in the tests currently used to identify potential carcinogens well illustrate the problems involved.

Because animal studies are expensive and take a long time much work has been done on developing reliable short-term tests for initial screenings. But in view of the large number of compounds jockeying for attention, pre-selection of chemicals to be submitted to the screen on the basis of structural and physicochemical resemblances to known carcinogens is an attractive proposition. This is the approach advocated by a group of workers at ICI and

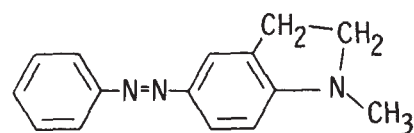
Alastair Hay is a Research Fellow at the Zoological Society of London.



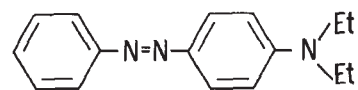
(I)



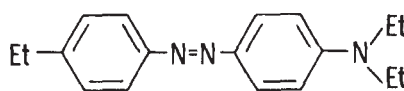
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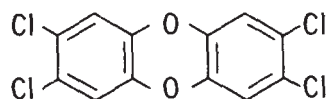
(III)



(IV)



(V)



(VI)

at the workshop John Ashby and his colleagues (ICI, Alderly Park) reported experiments testing the effects of structural modifications of butter yellow on its performance in one of the short-term tests.

Butter yellow gives a predominantly negative response in the Ames test (which tests the ability of a chemical to cause mutations in bacteria). It does, however, give a reproducible positive response in the Styles cell transformation test (*Br. J. Cancer*, in the press). The 3-methyl analogue (II) reported to be non-carcinogenic in animal studies proved negative in this test as well, as might be expected on theoretical grounds. The 3-methyl group forces a change in the angle of the NMe_2 group with respect to the benzene ring. This would reduce or abolish the stability of the nitronium ion thought to be involved in the binding of compounds such as (I) to DNA (Lin *et al. Cancer*

Res. **35**, 844; 1975). To check the importance of the angle change in the carcinogenic deactivation of (II) Ashby *et al.* synthesised and tested the indoline (III). Here the overall substitution pattern is the same, but by joining the 3-methyl group to one of the NMe_2 groups planarity between the nitrogen base pair of electrons and the benzene ring is restored.

In the Styles assay (III) was positive. So far no derivatives of the indoline have been tested *in vivo* for carcinogenicity, but these results suggest that it might be a liver carcinogen. If this turns out to be true it would strengthen the theoretical basis for the difference in activity between (I) and (II).

Curiously the NEt_2 analogue (IV) of butter yellow is reported to be non-carcinogenic, whereas the 4'-ethyl analogue (V) is a carcinogen. The small structural change involved in the addition of an ethyl group to (IV) would hardly be expected to reintroduce carcinogenicity if a fundamental principle were involved in the activity of (IV). Needless to say both (IV) and (V) were positive in the Styles assay. This suggests that either (IV) has been inadequately tested in animals for carcinogenicity, or that perhaps some nonspecific and secondary mechanism such as its solubility, or transport into the cell prevented it from producing tumours *in vivo*. The Styles assay clearly predicts (IV) as a potential carcinogen, and surely represents an efficient short-term test. But the Styles assay does fail to predict β -naphthylamine as a carcinogen whereas this compound produces a positive response with the Ames test. Clearly both tests are necessary for a screening net of finer mesh.

TCDD presents a more complex problem. It is a planar tricyclic aromatic compound (VI) said to be chemically similar to many polycyclic hydrocarbon carcinogens which bind to DNA directly or through the formation of a metabolite (Poland & Kende *Fedn Proc.* **35**, 2404; 1976), or to the acridines which intercalate in DNA (Hussain *et al. Ambio* **1**, 1; 1972). In fact the chemistry of TCDD is not like that of the known carcinogens. Whereas the carbon atoms which bridge the benzene rings in the tricyclic aromatic carcinogens form part of a conjugated bond system, this is not true of the oxygen atoms in TCDD. The oxygens do not conjugate to the benzene rings. From a structural point of view, therefore, TCDD would not be expected to be a carcinogen. The possibility exists, however, that ring epoxidation may occur *in vivo* to convert it into a potential carcinogen. But the metabolism of TCDD, if it does occur, is very slow (Piper *et al. Environ. Health Perspect.* **5**, 241; 1973).

From the evidence available it seems that TCDD is not mutagenic in all bacterial systems. It is reported to be mutagenic in *Escherichia coli* Sd-4 (Hussain *et al.*, *op. cit.*) and *Salmonella typhimurium* TA 1532 (Hussain *et al.*, *op. cit.*, Seiler, *Experientia* **29**, 662; 1975) but not in the *S. typhimurium* strain TA 1530 (Hussain *et al.*, *op. cit.*). Ames and Yamasaki (quoted in Poland & Kende, *op. cit.*) were unable to demonstrate reversion of specific mutant strains of *S. typhimurium* by TCDD treatment.

The only animal study to test the carcinogenic potential of TCDD so far reported is that of Van Miller and Allen (*Fedn Proc.* **36**, 396; 1977). These authors report that in rats TCDD causes carcinomas of the kidney, liver and skin as well as angiosarcomas. This is a disturbing claim which urgently requires confirmation. It is fortunate, therefore, that two other animal studies are nearing completion—those of the Dow Chemical Company and US National Cancer Institute. Their results are awaited eagerly.

It should perhaps come as no surprise that the clinical data on TCDD's potential mutagenic or carcinogenic properties are mixed. G. Reggiani (Hoffmann-La Roche, Basle) reviewed the clinical picture of 800 cases of presumed TCDD exposure in workers—all of whom developed chloracne—exposed to the chemical during the production of 2,4,5-trichlorophenol. The frequency of chromosomal abnormalities in these workers and their families is no greater than the statistical norm. As for the incidence of cancer, the evidence from two factory studies at the Spolana plant (Czechoslovakia) and BASF site (Germany) suggests that the rate is not increased in workers exposed to the dioxin.

Claims to the contrary come from Vietnam. An increase in liver cancer in individuals exposed to the herbicide 2,4,5-T in which TCDD is present as a contaminant may be attributable to the dioxin (Tung reported in Galston *Nature* **258**, 2; 1975). A preliminary study by Tung *et al.* (*Vietnamese Studies* **29**, 53; 1971) also reports an increased incidence of chromosomal abnormalities in the victims of herbicide spraying. The Vietnamese study has been criticised (*Effect of Herbicides in South Vietnam*, National Academy of Sciences, 1974) as being statistically inadequate. It is being repeated, but on a larger scale.

G. Tognoni (Mario Negri Institute, Milan) found the Vietnamese reports disturbing. Although no evidence of chromosomal abnormalities has so far been observed in the Séveso population he was far from optimistic about their long term health prospects.

There is obviously an urgent need

to resolve the question of whether or not TCDD is a potential carcinogen. The Van Miller and Allen study is the only one so far which claims that it is. Their claim needs to be supported by experiments in other animal species. Other short term tests could also be applied; the results from these could perhaps clear up the conflicting evidence. □

Degradative plasmids

from J. R. Saunders

MEMBERS of the bacterial genus *Pseudomonas* are renowned for their capacity to utilise an enormous range of compounds as energy and carbon sources. Over the past few years it has become clear that the ability of these organisms to degrade certain exotic substrates is specified by genes borne on plasmids. A variety of such 'degradative plasmids', some of which are self-transmissible, has been identified (see Chakrabarty *A. Rev. Genet.* **10**, 7; 1976). Thus plasmids can confer on host pseudomonads the ability to break down naphthalene (NAH plasmid), salicylate (SAI plasmid), camphor (CAM plasmid) *n*-octane (OCT plasmid) and toluene and xylenes (TOL plasmid).

TOL plasmids carry the genes encoding the degradation of benzoate and *m* and *p*-toluates by way of the *meta* (α -ketoacid) pathway in *P. putida*. A functional *meta* pathway is also specified by other degradative plasmids. It involves a single set of enzymes allowing growth on a wide range of hydrocarbons, aldehydes, alcohols and carboxylic acids, all of which may present themselves as potential sources of nutrition in the wild. Furthermore, the location of the genetic determinants for the *meta* pathway on plasmids increases versatility by assisting the spread of the degradative trait in the bacterial population. In common with many other saprophytic pseudomonads, *P. putida* possesses an alternative route for dissimilation of benzoate, the *ortho* (β -ketoacid) pathway. The *ortho* pathway is chromosomally determined and like the *meta* pathway initially involves the oxidation of benzoate to catechol. Subsequently the pathways diverge: the *ortho* pathway requires the conversion of catechol to *cis-cis* muconate by catechol 1,2 oxygenase, the product in this case being the inducer for the enzyme. In contrast benzoate itself probably initiates expression of the *meta* pathway by inducing catechol 2,3 oxygenase which

converts catechol to hydroxymuconic semialdehyde. Consequently when the genes for both pathways are present, as in a Tol⁺ strain of *P. putida*, benzoate is preferentially degraded by the plasmid-coded *meta* pathway and not the chromosomal *ortho* pathway. way.

Ironically Mike Worsey and Peter Williams (*J. Bact.* **130**, 1149; 1977) have found that some strains of *P. putida* grow faster on benzoate when using the *ortho* pathway. Thus growth of such strains on benzoate exerts a selective pressure for loss of Tol function. This may be by elimination (curing) of the entire 78 Mdal TOL plasmid or by deletion from the plasmid of a specific segment of about 27 Mdal which encodes all or part of the TOL pathway (Bayley *et al. Molec. gen. Genet.* **154**, 203; 1977). Growth on benzoate can also select for strains which have an intermediate (B3) phenotype. These can still grow on toluene, *m*-xylene and benzoate but are incapable of using *m*-toluate. Strains of the B3 phenotype apparently possess a regulatory mutation which blocks induction of *meta* pathway enzymes by benzoate and *m*-toluate but enables induction by toluene and *m*-xylene. It is envisaged that early enzymes for utilisation of *m*-toluate and benzoate are positively controlled by two separate regulators, one of which interacts with the inducers toluene and *m*-xylene, whereas the other (lacking in strains of B3 phenotype) interacts with benzoate and *m*-toluate. Further studies on the genetic regulation of this and other degradative pathways promise to be of great interest.

Peter Williams and coworkers at the University of Wales, Bangor, in collaboration with Paul Broda's group at the University of Edinburgh have also characterised the plasmids in thirteen Tol⁺ pseudomonads isolated from soil samples in different geographical locations (Duggleby *et al. J. Bact.* **130**, 1274; 1977). They have assigned the TOL genes to a number of plasmid species in different organisms. Interestingly five of these plasmids are indistinguishable on the basis of their molecular weights (all about 77 Mdal) and gel electrophoresis profiles after endonuclease digestion. One member of this apparently homologous series of plasmids was resident in a strain isolated in Japan whereas the other host strains were found in Wales. This implies widespread distribution of a single TOL plasmid. Although worldwide spread of particular R plasmids is well documented and explicable in terms of international travel and human contact, it is harder to see how a single degradative plasmid could become broadcast amongst the population of soil bacteria. One possibility

would be the spread of carrier strains by large scale import and export of industrial materials such as oil products contaminated with soil bacteria.

Degradative plasmids obviously contribute greatly to the already substantial nutritional versatility of *Pseudomonas* species. Many of the compounds degraded under the control of such plasmids would otherwise be toxic to other forms of life. Such degradation is therefore of some importance in detoxifying localised areas of pollution. Indeed it has been proposed that *Pseudomonas* strains carrying degradative plasmids be used to disperse oil spillages and render them harmless. Since most of the degradative plasmids belong to different incompatibility groups it is possible to construct pseudomonads which carry a battery of different degradative plasmids which together confer enhanced ability to utilise crude oil. Chakrabarty, who has been a prominent advocate of this approach, has pointed out that such strains would also have to harbour mercury-resistance plasmids to cope with the high levels of the heavy metal in oil residues.

The deliberate introduction of such constructed strains into the environment is open to the same kinds of objections as those levelled against recombinant DNA research. Such ventures could rebound if for example they led to the breakdown of petroleum products in storage tanks or the induction of pathogenic characteristics in previously harmless saprophytic species. However initial studies on the 'epidemiology' of TOL plasmids indicate that the population of soil bacteria has itself already responded to the increasing load of organic pollutants that has leaked into the environment. Selective forces, analogous to those that have led to the spread of antibiotic resistance in clinically important bacteria, are probably operating to produce bacteria that are resistant to and able to degrade industrial pollutants. In this case the consequences for mankind should be beneficial. □



A hundred years ago

At the Guy's Hospital *conversazione*, on Monday evening, a new government filter, invented by Major Crease, was shown which reduced strong tea and infusions of logwood to clear tasteless water. The nature of the filtering material is not made known.

From *Nature* **16**, 48; 4 October; 1877.

review article

Thresholds and breakpoints in ecosystems with a multiplicity of stable states

Robert M. May*

Theory and observation indicate that natural multi-species assemblies of plants and animals are likely to possess several different equilibrium points. This review discusses how alternate stable states can arise in simple 1- and 2-species systems, and applies these ideas to grazing systems, to insect pests, and to some human host-parasite systems.

IN all but the most trivial areas of enquiry, there arise questions about the extent to which events are shaped by predictable natural laws as against the accidents of initial conditions and perturbations. Is the human story largely a deterministic tale of civilisations marching to Toynbee's tune, three and a half beats to disintegration, or did the hinge of history turn on the length of Cleopatra's nose? Such questions of the relative roles of chance and necessity¹ are fundamental in modern cosmology^{2,3}, in the foundations of statistical mechanics^{4,5}, and in evolutionary biology¹ and ecology, even though they may arise in less blatant and romantic fashion than the 'what ifs' of history and the social sciences.

Viewing the grand sweep of evolution, we can see many examples where the taxonomic details of the plant or animal that occupies a given niche at a given time and place depend on historical accident, but where the niches themselves, and the broad patterns of community organisation, are remarkably constant⁶⁻⁹.

Taking a much narrower and more local view, it is interesting to consider a particular assembly of species, with specified interactions among them, and to ask questions about the dynamics of the system. Is the dynamical behaviour described by the multi-dimensional generalisation of a single valley (a global attractor)? Or is the dynamical landscape pockmarked with many different valleys, separated by hills and watersheds? If the former, the system has a unique stable state, to which it will tend (like a marble seeking the bottom of a cup) from all initial conditions, and following any disturbance. If the latter, the state into which the system settles depends on the initial conditions; the system may return to this state following small perturbations, but large disturbances are likely to carry it into some new region of the dynamical landscape (so that the system behaves rather like the ball in a pin-ball machine). If there is a unique stable state, historical effects are unimportant; if there are many alternative locally stable states, historical accidents can be of overriding significance. Obviously, questions of this kind are very important in the understanding and management of ecosystems.

A large body of empirical observations shows that many natural communities have a multiplicity of stable states. Sutherland¹⁰ has demonstrated that for the fouling community (a complex assembly of hydroids, tunicates, bryozoans, sponges and associated species) at Beaufort, North Carolina, the order of larval recruitment determines the way the community develops. Reviewing other work on the marine rocky intertidal (see also the review by Levin¹¹), on coral reefs, on freshwater lakes, and on terrestrial plant communities, Sutherland concludes that community structure can often "be explained only by referring to specific historical events" and therefore that "multiple stable points are an undeniable

reality." A similar conclusion emerges from Connell's and Slatyer's¹² survey of mechanisms of succession in natural communities.

The view that complicated ecosystems possess many alternative stable states is also supported by theoretical studies of mathematical models that caricature such systems. From the growing number of possible examples, I mention only two, chosen from opposite ends of the spectrum. Austin and Cook¹³ have made computer studies of a system in which 94 species (embracing plants, herbivores and carnivores) are linked together by interactions that aim to be relatively realistic; the system has many equilibrium points, and is easily transferred from one to another. Case and Gilpin¹⁴ have explored a relatively abstract system, in which the coefficients in the interaction matrix for a n -species Lotka-Volterra model are assigned random values; if n is at all large, the system typically collapses to one or other of a variety of simpler systems with fewer species, and this final steady state depends on the initial population values. The notion of 'resilience' has been introduced by Holling¹⁵ in an attempt to characterise the degree to which a system can endure perturbations without collapsing or being carried into some new and qualitatively different state. Theoretical ideas about resilience, along with interpretive reviews of diverse other meanings that can be attached to 'stability' in an ecological context, are the subject of many recent papers¹⁶⁻²².

It is thus clear that real ecosystems possess multiple stable states, as do plausible mathematical models. Unfortunately, the complications inherent in multi-species systems almost invariably preclude any quantitative confrontation between theory and data. For multi-species communities, the empirical observations remain largely anecdotal, and the theory remains largely metaphorical.

For simple 1- and 2-species situations it is, however, beginning to become possible to put theory and observation together, to gain insight into the workings of systems with more than one stable state. This review is a synthesis of several examples of this kind. I begin with grazing ecosystems (and then, more generally, any harvested crop or animal population), go on to insect pest systems (particularly the spruce budworm), and conclude with some human host-parasite systems.

Grazing ecosystems

Consider^{23,24} a population of herbivores, maintained at a constant density H , and sustained by vegetation whose biomass is V .

Our interest is centred on the dynamics of the vegetation biomass. Following Noy-Meir²³, suppose that in the absence of grazing the growth rate of the vegetation as a function of V is $G(V)$, and that the herbivores consume the vegetation at a net rate $C(V)$ (corresponding to a per capita consumption at a rate $c(V):C(V)=Hc(V)$). Then the rate at which V changes is given by

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$$dV/dt = G(V) - C(V) = G(V) - Hc(V) \quad (1)$$

The vegetation biomass will thus tend to settle to an equilibrium level where the natural growth rate exactly balances the loss rate due to grazing; that is, to a value of V such that $G(V) = C(V)$. Noy-Meir shows how these equilibrium V -values can be found graphically, for various assumptions about the way $G(V)$ and $C(V)$ depend on V .

Fig. 1 illustrates one plausible situation. Here, as shown by the solid curve, $G(V)$ is positive even when $V = 0$ (corresponding to some constant background contribution to the vegetation growth rate, for example from the sprouting of seeds blown in from other areas). At first $G(V)$ increases as V increases, but as V continues to increase $G(V)$ decreases as shading and competition for nutrients becomes important, until $G(V) = 0$ at $V = K$; K is the maximum stable biomass of ungrazed vegetation. The general shape of the per capita consumption function $c(V)$ is a saturation curve: at low V , the herbivore intake is limited by forage availability, so that $c(V)$ increases with increasing V ; at high V , $c(V)$ saturates to some constant determined by the animal's intake capacity or digestion rate. It is useful to christen V_0 as the characteristic value of V at which the consumption function saturates. The dashed curves for total consumption rate $C(V) = Hc(V)$ in Fig. 1 are for a $c(V)$ that increases linearly with V at small V , corresponding to a herbivore that searches randomly (Holling's "Type II" or "invertebrate" predator search pattern²⁵).

We see from Fig. 1 that for relatively small values of H there is a single equilibrium value for V , given by the point A where $G(V)$ and $C(V)$ curves intersect; this value is slightly less than K . For relatively large values of H , there is again a unique equilibrium value for V (corresponding to the point E), now at a low value of V . For intermediate values of H , the $G(V)$ and $C(V)$ curves intersect at three points. The points B and D correspond to locally stable V -values, whose domains of attraction are divided by the unstable equilibrium point corresponding to C; B and D are the bottoms of adjacent valleys, and C marks the watershed between them.

The essential feature that leads to two alternative stable states for intermediate H values in Fig. 1 is the assumption that $c(V)$ saturates to a constant for values of V significantly below the ungrazed equilibrium. That is, defining

$$\alpha = V_0/K \quad (2)$$

Fig. 1 is for α significantly less than unity. If α exceeds, or is of the

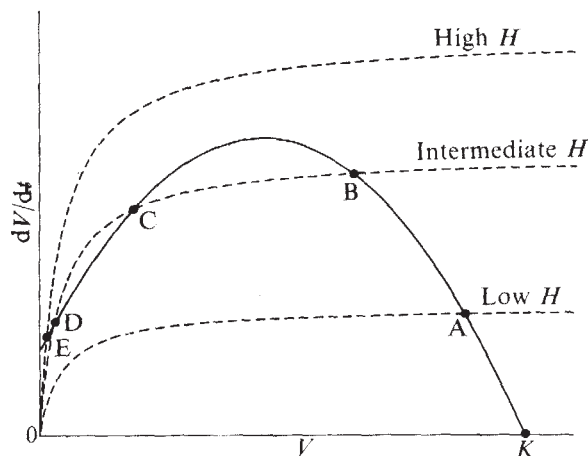


Fig. 1 The rate of change of vegetation biomass, dV/dt , is shown as a function of V . The solid curve is the natural, ungrazed vegetation growth rate (which here is finite even for $V = 0$). The dashed curves are loss rates due to grazing (of Type II pattern) at high, intermediate and low herbivore densities, H . Where the solid curve lies above the dashed one, the net growth rate is positive; where the solid curve lies below the dashed one, the net growth rate is negative; the points of intersection of the curves correspond to possible equilibrium points. For further discussion, see the text.

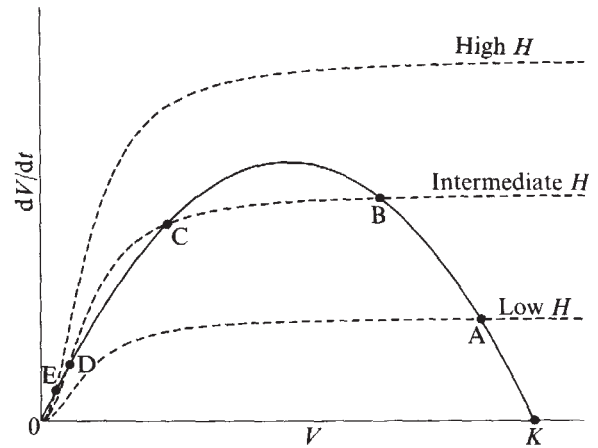


Fig. 2 As for Fig. 1, except that here the natural growth rate (solid curve) is linearly proportional to V at small V , and the loss rate due to grazing (dashed curves) is of Type III. Specifically, this figure corresponds to equations (3) and (4); the α of equation (2) is here $\alpha = 0.1$, and 'high, intermediate and low H ' are represented by $\gamma = 0.35, 0.22$ and 0.10 , respectively. The basic features shown by the figure are, however, generic.

order of, unity, there is always only one stable state in the figure corresponding to Fig. 1.

Fig. 2 illustrates another plausible circumstance, which differs from Fig. 1 in details but not in essentials. Here, as V increases, $G(V)$ increases from zero to some maximum value and then decreases back to zero at $V = K$. The per capita consumption function $c(V)$ is now for herbivores whose foraging efficiency increases faster than linearly with V at low V values, but which again saturates to a constant for V above some characteristic value V_0 (Holling's "Type III" or "vertebrate" predator consumption function²⁵, which is also typical of many invertebrates²⁶⁻²⁸). Provided that V_0 is significantly less than K (that is, α is small), we can again distinguish three domains of dynamical behaviour for the vegetation biomass: for small H there is a unique equilibrium value of V , slightly below K (the point A); for large H there is a unique equilibrium at a low V value (the point E); for intermediate H there are two alternative stable states (the points B and D), divided by an unstable point (C).

Specifically, Fig. 2 illustrates the situation where $G(V)$ is given by the familiar logistic equation, $G(V) = rV(1 - V/K)$, and where $C(V)$ is the "Type III" consumption function $C(V) = \beta HV^2/(V_0^2 + V^2)$. Then equation (1) has the particular form

$$\frac{dV}{dt} = rV \left(1 - \frac{V}{K}\right) - \frac{\beta HV^2}{V_0^2 + V^2} \quad (3)$$

This equation may equivalently be written in dimensionless form, by using the earlier definition of α , and introducing the rescaled variables $X = V/K\tau = rt$ and $\gamma = \beta H/rK$

$$dX/d\tau = X(1 - X) - \gamma X^2/(\alpha^2 + X^2) \quad (4)$$

This equation can exhibit (D. Ludwig, D. Jones and C. S. Holling, to be published) two alternative stable states if, and only if, $\alpha < 1/3, 3$.

Figure 3 is derived from the situation depicted in Fig. 2, and shows the stable equilibrium value(s) of the vegetation V , as a function of the herbivore density H . For low H , the vegetation biomass tends to settle to a unique steady value, slightly below K . As H increases beyond a threshold value (T_1), a second stable state for V appears, in a discontinuous fashion. As H continues to increase, there occurs a second threshold (T_2), beyond which there is again only one stable equilibrium for V (the original low- H state having disappeared, discontinuously). For stocking densities in the intermediate region, $T_1 < H < T_2$, the vegetation will tend

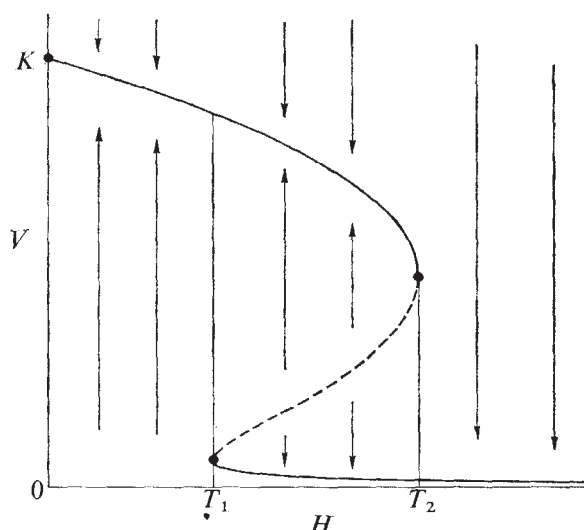


Fig. 3 The equilibrium values of the vegetation biomass, V , are shown as a function of the stocking density, H . For a fixed value of H below the lower threshold at T_1 , or above the upper threshold at T_2 , there is a unique equilibrium value of V ; any initial V value will move to this equilibrium, as indicated by the arrows. For H between T_1 and T_2 , there are two alternative equilibria for V ; as shown by the arrows, the system will move to the upper or lower equilibrium, depending on whether the initial value of V lies above or below the dashed 'breakpoint' curve. (This curve is constructed from Fig. 2 and equations (3) and (4), with $\alpha=0.1$.)

toward either the upper or the lower equilibrium value, depending on the initial value of V : for initial values lying above the dashed line in Fig. 3, V will move toward the upper equilibrium; initial values below the dashed line will move to the lower equilibrium. Borrowing an epidemiological term²⁹, we may call the dashed line the locus of the "breakpoint" values of V .

Although Fig. 3 was constructed from the specific equation (3), its important features are generic to any grazing ecosystem where the dynamics of the vegetation biomass is described by something like Figs 1 or 2. Even in the specially simple case where the $G(V)$ of Fig. 2, with $G(0) = 0$, is combined with the $C(V)$ of Fig. 1, with a linear dependence on V for small V , we still obtain Fig. 3, except that now the lower equilibrium value (occurring for $H > T_1$) is $V = 0$.

If it is assumed that gross animal production, P_G , is linearly proportional to total consumption, $C(V)$, then the equilibrium level of P_G as a function of stocking density H can be read off from Fig. 3. This is done in Fig. 4. Fig. 4 has all the properties just adumbrated for Fig. 3, and is similarly generic.

Noy-Meir first points out some management morals implicit in all this, and then discusses the extent to which such theoretical insights accord with known facts.

If one has a grazing system capable of manifesting the discontinuities illustrated in Figs 3 and 4, then the vegetation will tend always to recover to a high level following an environmental disturbance only if H is kept below some threshold density, T_1 . But, as can be seen from Fig. 4, this usually implies unacceptably low gross productivity. Conversely, for H above T_1 , there is the danger that an environmental fluctuation will carry V below the breakpoint value (the dashed line), whereupon the system will move into the alternative steady state, with dramatically lower values of V and P_G . The closer the system is pushed toward the point of maximum productivity (at $H = T_2$), the more likely is this discontinuous collapse. And of course if the stocking rate for maximum productivity is misjudged, so that H is pushed beyond T_2 , then the system must collapse to the lower equilibrium state. As Noy-Meir²³ sums it up, "a discontinuously stable system is highly labile at the stocking rate which allows the highest productivity. Such a grazing system can be maintained at or near this maximum production rate only by very frequent, almost constant, adjust-

ments of stock density in response to fluctuations in vegetation. A somewhat lower stocking rate will ensure more stable, though on the average somewhat lower, production."

Noy-Meir reviews two classes of empirical evidence.

For intensive, or pasture, systems he pulls together data on the growth curves as a function of green biomass, $G(V)$, for ryegrass-clover in New Zealand^{30,31} and for clover in Australia³², and on the consumption curves for sheep, $C(V)$, for *Phalaris*-clover³³, for *Phalaris*-annuals-clover³⁴, and for ryegrass-clover³⁵, all in Australia. He concludes that the sheep tested by Arnold and by Willoughby would be capable of discontinuous stability properties in October (down under) in pastures of ryegrass, ryegrass-clover, and possibly in clover. There is also direct evidence for two alternate stable states in an experiment done by Morley³⁶, which involves sheep at three different stocking rates in two grazing systems (continuous and with rotation), with three replicates of each.

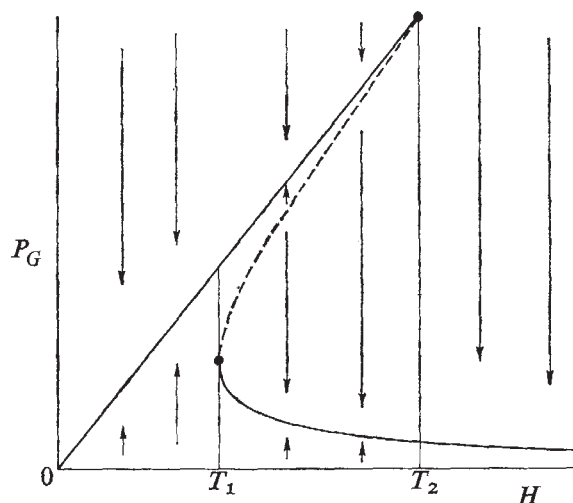
For extensive, or range, systems, indirect evidence can be culled from traditional management practices. Noy-Meir lists the conventional distinction between 'safe' and 'maximum' stocking rates, the notion of range readiness (allowing the vegetation to build to some pre-determined level before introducing animals), and the observation that productivity and animal condition may remain high even though the range is on the verge of collapse. This 'conventional wisdom' is readily justified by Figs 3 and 4.

Throughout this discussion, the herbivore density H has been treated as a constant. In natural (as opposed to managed) situations, H itself will be a dynamical variable, whose rate of change depends on V and H . The consequent pair of differential equations for $H(t)$ and $V(t)$ have been discussed by many people: see the review by Caughley²⁴. The system may have a unique stable point, or two (or even more) alternate stable points, or the populations may oscillate in a stable limit cycle. This subject will be pursued further, when we come to the budworm model, below.

Harvesting animal populations

In grazing ecosystems, we considered the dynamical behaviour of a population (the 'vegetation', $V(t)$) which was being harvested by herbivores. Much of the discussion can be translated to apply to other systems where a plant or animal population is harvested, either directly or indirectly, by man. In particular, fisheries provide an example where one is interested in the dynamics of a fish population, and where the net population growth rate involves a natural growth term, $G(V)$, and a loss rate due to harvesting, $C(V)$, which in general depends both on the fishing effort and on the fish

Fig. 4 Gross animal productivity, P_G , is shown as a function of H . This curve is derived from Fig. 3, under the assumption that P_G is proportional to the total consumption, $C(V)$, of equation (1). The main features are as in Fig. 3; for further discussion, see the text.



population density. The same is true of whaling industries, and of commercial forestries.

Brauer and Sanchez³⁷ have considered a fish or other population that is harvested to give a constant yield. Their analysis corresponds to Fig. 2 in the limit $\alpha \rightarrow 0$; that is, they have the $G(V)$ curve of Fig. 2, but their consumption or harvesting curves are purely horizontal lines, already saturated at $V = 0$. The resulting equilibrium fish population (V) as a function of yield (H) is a limiting version of Fig. 3, with T_1 at the origin, and the lower equilibrium curve collapsed to the H axis ($V = 0$).

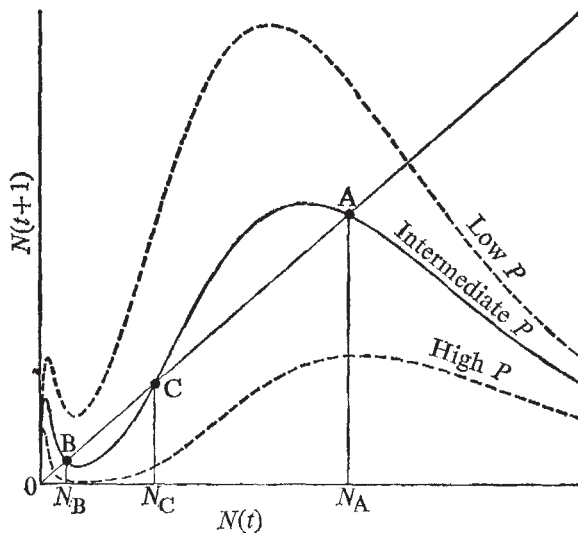


Fig. 5 This figure is a difference equation version of Fig. 3, for animals with non-overlapping generations. The curves relating the population magnitudes in successive generations are shown for various levels of predation, P . Possible equilibrium points occur where the curves intersect the 45° line. (Specifically, the difference equation used here is $N(t+1) = N(t) \exp[r\{1 - N - PN/(x^2 + N^2)\}]$, with $x = 0.1$, $r = 4$, and $P = 0.35, 0.22, 0.15$.)

A more realistic harvesting curve will acknowledge that, at low fish population values, the effort needed to keep the yield constant is prohibitively high. Consequently, a realistic curve for attempted 'constant yield' harvesting will be more like those in Fig. 1 and 2, and much of the discussion of the system's dynamics (ref. 38 and J. R. Beddington, to be published) parallels that by Noy-Meir. Beddington and May's³⁸ study is focused on the response of the system to environmental fluctuations, and to this end employs differential equations with stochastic coefficients. Nevertheless, the qualitative conclusions of this relatively sophisticated study (especially the caution against trying to maximise sustained yield under a strategy of constant quotas, which is roughly equivalent to operating around T_2 in Fig. 4) are laid bare by the simple and deterministic graphical analysis outlined above.

Other recent studies of harvested systems which can have alternate stable states are due to Goh³⁹ and to Huberman⁴⁰ (who uses equation (3)). Clark⁴¹ has given a fine review.

Insect pests: general remarks

We now alter our viewpoint, to think of $V(t)$ more generally as a 'prey' population. Its dynamical behaviour depends on the trophic level above it (the 'predators', which were the herbivores H in the grazing systems) and on the trophic level below it (the resources, which were the light and nutrients determining K in the grazing systems). At the risk of confusing the reader, I shall symbolise this change of viewpoint by using $N(t)$, to replace $V(t)$, for the prey population, and by using P , to replace H , for the predators.

In dealing with insect pests, our attention has shifted up one rung in the trophic ladder. Instead of the vegetation $V(t)$ which

depends on a variable H and a constant K , we have a population $N(t)$ of herbivorous insects which depends on predators P and vegetational resources K , either of which may vary.

For a given value of the environmental carrying capacity (K) and for a specified density of predators (P , formerly H), the considerations that determine the dynamics of the prey population (N , formerly V) are likely to be as in Fig. 2. That is, the growth rate of the prey population may be typified as logistic, and the predators' attack rate will have the general form shown in Fig. 2 both for vertebrate²⁵ and for many invertebrate²⁶⁻²⁸ predators.

One final complication must be disposed of. For the majority of temperate zone insects, population growth is a seasonal (often annual), rather than a continuous, affair. Thus the continuous rate of population change, dN/dt , should usually be replaced by discrete changes, $N(t+1) - N(t)$, at time intervals one unit (often one year) apart. The relation between the population values in successive generations will still typically be determined by Fig. 2, but with $N(t+1) - N(t)$ replacing dN/dt on the y-axis. It is more traditional, in this case, to plot the curve that relates $N(t+1)$ to $N(t)$. This is done in Fig. 5. The 45° line represents population values that are unchanging from one generation to the next, and therefore the points where the curves intersect this line are possible equilibrium states. Where the solid curve lies above the dashed curve in Fig. 2, the net growth rate is positive, and the curve lies above the 45° line in Fig. 5. Conversely, where the solid curve lies below the dashed one in Fig. 2, the curve lies below the 45° line in Fig. 5.

When predation is relatively unimportant (low H in Fig. 2, low P in Fig. 5), there is a unique equilibrium point, at a population value slightly below K ; the population level is set primarily by resources. When predation is relatively important (high H in Fig. 2, high P in Fig. 5), there is again a unique equilibrium point, this time at a low population level; the population is predator-controlled. But for intermediate levels of predation there are, as before, two attracting equilibrium points (A and B in Fig. 5), divided by a repelling point (C in Fig. 5).

The consequences of this intermediate level of predation, with its two alternative equilibria for the pest population, have been noted by Takahashi⁴² and Watt⁴³. They observe that such pests may usually fluctuate at low numbers, around the lower equilibrium point at N_B . But if fluctuations in the number of predators, or of pests, or of the carrying capacity, happen to carry pest numbers above N_C in any one year, then the population will explode towards the upper equilibrium point at N_A . A subsequent crash is likely (the complications that make the dynamics of difference equations more unsteady than those of differential equations are discussed in the conclusion), with the population returning to the neighbourhood of N_B . The pest population may thus exhibit a periodic or episodic pattern of 'outbreaks', followed by relatively long intervals at low densities.

Southwood⁴⁴⁻⁴⁶ has recently given a more quantitative discussion, and he and others have incorporated the practical conclusions into a morphology of strategies appropriate for the control of various kinds of insect pests⁴⁷⁻⁵⁰.

For example, Southwood and Comins⁴⁴ have interpreted Clark's thorough studies of the eucalyptus psyllid (a plant louse) *Cardiaspina albipunctata* in the light of Fig. 5. Using Clark's data for egg-to-adult survival for 29 generations, along with information about the depression of natality by direct crowding and by host plant deterioration, they estimate the shape of the $N(t+1)$ -versus- $N(t)$ curve. They find the curve to be of 'intermediate P ' type, and assign to N_B , N_C and N_A values corresponding to 2.8, 22 and 107 eggs per shoot, respectively. Not only does this give a qualitative explanation of the observed episodic outbreaks, but it is in remarkable quantitative agreement: Clark's field data give an average of 3.0 eggs per shoot at low densities, suggest a 'rapid increase to outbreak level' beyond 10-15 e/s, and have a mean for 12 high density populations of 110 e/s (with a range 23-280).

Sufficient data have also been published on the European spruce sawfly, *Diprion hercyniae*, in New Brunswick for a tentative analysis of this kind. Southwood⁴⁷ suggests the present situation is one of 'intermediate P ', with the sawfly controlled by a com-

bination of virus disease and parasitoids: life-table estimates and field observations agree on a value of N_c around $0.2 \text{ larvae m}^{-2}$; fluctuations to higher densities result in runaway to around $N_A \sim 1 \text{ larvae m}^{-2}$, which is economically acceptable. Before the advent of the virus disease, the sawfly in New Brunswick seems to have been⁴⁷ in a "low P " situation, with outbreaks to densities around 20 larvae m^{-2} followed by crashes.

Insect pests: the spruce budworm

One of the best studied of all insect pests is the spruce budworm, *Choristoneura fumiferana*, in Canada. This forest defoliator erupts at approximately 40-year intervals, causing much damage in northern coniferous forests, and economic stress in the lumber industry. The massive amount of data gathered by Morris⁵¹ and his associates has been subjected to much analysis by Holling and his collaborators^{15,52} (and D. Ludwig, D. Jones and C. S. Holling, to be published) at UBC and at the International Institute for Applied Systems Analysis (IIASA). One of the most pleasing features is the way the models have become progressively simpler as the basic mechanisms have become better understood. Thus the earlier 'systems models' have given way to Ludwig *et al.*'s⁵³ 3-component model (budworms; average leaf area per tree; energy reserve determining the condition of trees and foliage); the present review gives a crude 2-component model (budworms; leaf area) that retains the essentials.

Consider first the dynamics of the budworm population, $N(t)$. Yet again, this is plausibly described by Fig. 2 (with N replacing V), with the net population growth rate counterpoised between a natural growth term and losses due to predators. The general features described by Fig. 2 may be typified by the specific equation (3), which now reads

$$\frac{dN}{dt} = rN \left(1 - \frac{N}{K} \right) - \frac{\beta P N^2}{N_0^2 + N^2} \quad (5)$$

Here r is the intrinsic growth rate for budworms, and K is the carrying capacity, which depends on the average leaf area per tree, S ; we write $K = \kappa S$. N_0 is the characteristic budworm population at which the predator attack rate saturates (to the constant level β per predator). This characteristic value for predator switching will usually depend on budworm density per unit leaf area rather than on absolute budworm numbers, so that we may write $N_0 = \eta S$. Finally, for a given value of average leaf area S , it is convenient to use the rescaled variables $X = N/\kappa S$, $\tau = rt$ and $\gamma = \beta P/r\kappa S$ to rewrite equation (5) in dimensionless form as

$$dX/d\tau = X(1-X) - \gamma X^2/(x^2 + X^2) \quad (6)$$

Here x is formally defined as $x = \eta/\kappa$, but biologically it retains the meaning it had in equations (2) and (4): for any given value of S , x is the ratio between the budworm density that saturates the predator attack capacity and the maximum budworm density that the vegetation can sustain. Equations (4) and (6) are identical.

As before, it is clear, either from the general Fig. 2 (which is drawn for small x) or from the specific equation (6) (provided $x < 1/3 \sqrt{3}$), that the budworm system may have two alternative stable states. If we fix the resource level S , and plot the stable budworm density N as a function of the number of predators P , we get exactly Fig. 3 (with N replacing V , and P replacing H).

It is more interesting, however, to fix the predator level P and consider how the equilibrium value(s) of N varies with S . This can be done graphically from Fig. 2, or algebraically from equation (5). The result is Fig. 6. A simpler way to arrive at Fig. 6 from Fig. 3 is to notice, from equation (6), that the equilibrium value(s) of N/S depends only on γ , once x is fixed. But $\gamma = \beta P/r\kappa S$, so that S plays a part exactly like $1/P$: small P is equivalent to large S , and vice versa, whence Fig. 6 (with S for the x axis) follows from Fig. 3 (with P or H for the x axis). Moreover this makes biological sense. For budworms, the good life is few predators or lots of food; low P or high S . The bad life is the converse.

The main features of Fig. 6 need no elaboration. At low values of

S , there is a unique, and low, budworm density; the system is under predator control. At T_2 a second stable state appears discontinuously, and, for $T_2 < S < T_1$, N will tend to one or other of the two stable states, depending on which side of the 'breakpoint' locus it starts from. For S above T_1 there is again a unique stable state, in which the predators play little part.

This discussion of the budworm dynamics is not sufficient for a full elucidation of the way the interactive system of budworms and forest foliage behaves. We need also to consider how the average leaf area per tree, S , depends on N .

The essentials of such a discussion can be carried out⁵³, independent of any further details, by noting that the characteristic time scales for changes in N and in S are very different. The time scale for budworm population growth is months, or even weeks. The basic time scale for change in the average leaf area per tree depends on average branch area, and thence on the time scale for tree growth, which is typically measured in decades. Thus N changes on a relatively fast time scale, S on a slow one.

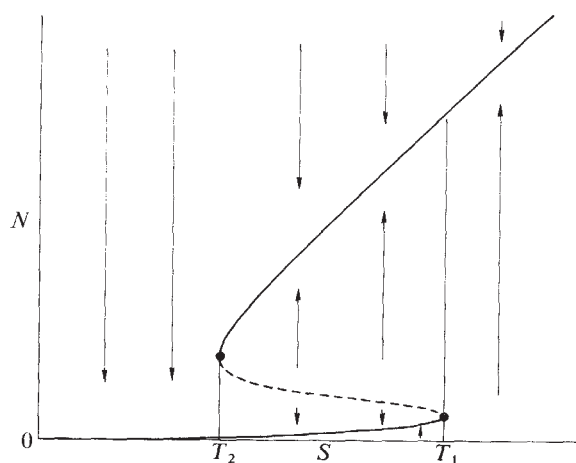


Fig. 6 The equilibrium budworm population, N , shown as a function of the average leaf area per tree, S . (This figure is based on equation (6), with $x = 0.06$.) The threshold and breakpoint features are similar to those in Fig. 3, and are discussed more fully in the text.

Returning to Fig. 6, we further suppose that budworm populations along the lower, 'predator-controlled' equilibrium curve do not have a significant impact on the foliage, so that S undergoes slow natural growth in this regime. Conversely, we assume that the large budworm populations along the upper equilibrium curve have a significant adverse effect on S , decreasing it. The qualitative nature of the system's dynamical behaviour now follows. Starting from any low values of N and S , the system will move rapidly (on the fast time scale) on to the lower budworm equilibrium curve in Fig. 6. S will now slowly increase, with N always quickly adjusted to the current S value, and the system will move (on the slow time scale) to the right along the lower equilibrium curve. When the system arrives at the point T_1 , no further continuous change is possible, and N jumps (on the fast time scale) to the upper equilibrium branch. Now S decreases because of the uncontrolled depredations of the budworms, and the system moves (fairly fast) to the left on the upper equilibrium curve. Finally, when the point T_2 is reached, N must fall (again on the fast time scale) back to the lower equilibrium branch, and the cycle begins again.

The upshot is a cyclic pattern of budworm explosions and crashes, as shown in Fig. 7. The system spends most of the cycle at low budworm densities, traversing the lower equilibrium curve from T_2 to T_1 . The cycle length is thus set by the slow time scale, and is of the order of decades.

A more explicitly mathematical treatment can be given crudely by assuming that the rate of change of S is given by a natural logistic growth term (with an intrinsic growth rate ρ), offset by

losses linearly proportional to the budworm density

$$dS/dt = \rho S(1 - S/S_{max}) - \epsilon N. \quad (7)$$

Under the assumptions discussed above, the locus of equilibrium values of S (the points where $dS/dt = 0$) are as indicated by the dashed line in Fig. 7. The pair of equations (5) and (7) then lead to a stable limit cycle, as shown in Fig. 7. The limit $\rho \ll r$ corresponds to the foregoing distinction between slow and fast time scales, and leads to a limit cycle with the almost discontinuous character that was discussed above.

The work of Ludwig *et al.*⁵² is more detailed (involving three

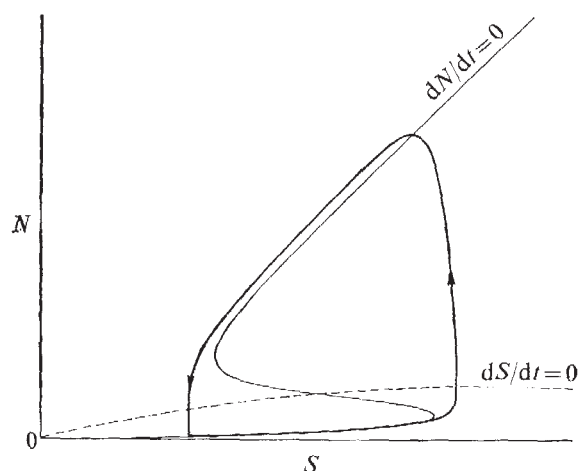


Fig. 7 The (solid, $dN/dt = 0$) equilibrium curve for budworms, N , as a function of foliage, S , is as in Fig. 6. The (dashed, $dS/dt = 0$) equilibrium curve for S as a function of N has the form of equation (7). Under these circumstances, the system will settle to oscillate in a stable limit cycle, as indicated. The figure is for $\rho = 0.01r$, so that the budworm growth time scale is fast compared with the time scale for tree growth: as explained more fully in the text, this explains the main features of the cycle.

differential equations), and is compared with field data. They obtain numerical agreement with the observed 40-year cycle.

This model has implications for the management of budworm populations. Notice first that a program aimed at stopping budworm outbreaks has a propensity to hold the population perpetually poised on the brink of explosion, around the threshold point T_1 on the lower equilibrium curve in Fig. 6. More quantitatively, the effects of using insecticide against budworms can be mimicked by adding an extra mortality term, $-sN$, to the right hand side of equation (5). If we could maintain $s > r$ for many years, the budworms could in principle be eradicated. In the more likely event that $r > s$, the new version of equation (5) can again be brought into the dimensionless form of equation (6), with α replaced by $\alpha' = (r/r - s)\alpha$. We recall that the kinkiness in Figs 3, 4 and 6, and the ensuing alternative stable states, depends on α being small. Thus use of insecticides can increase the effective value of α to such an extent as to straighten out the kink in Fig. 6 (that is, can increase α' to a value $\alpha' > 1/3\sqrt{3}$), leading to a unique stable state. The price, however, is daunting: not only are we committed to endless insecticide use, but the new equilibrium is necessarily established at a budworm density somewhere intermediate between the original upper and lower equilibrium levels. Such a budworm density represents a perpetual low-level outbreak.

It should be added that this discussion has ignored climatic fluctuations. One argument in favour of trying to 'hold the line' with insecticides is that one may thereby buy time, waiting for a year when unfavourable weather will carry the population back down to very low values.

Human host-parasite systems

Similar threshold and breakpoint phenomena occur in the transmission dynamics of many infections of humans and other

animals, particularly when the transmission cycle involves intermediate vectors.

Malaria is probably the best-known example exhibiting a threshold^{54,55}. Suppose that a single malarious mosquito is introduced into a closed and previously malaria-free community of people and mosquitoes. Each person bitten by this mosquito may, with a probability that can be estimated, become victim to the disease. These infected people may in turn be bitten by uninfected mosquitoes, which thereby (again with some probability factor, and after the elapse of a latent period of around 10 days) are recruited as malaria vectors. The key question is whether the original infectious mosquito produces, on the average, more than one subsequently infectious mosquito, or not. If it does, the system is 'above threshold', and the fraction of the human and of the mosquito populations that have malaria at any one time will grow until an equilibrium is reached, at which new infections are balanced against recoveries and deaths. Conversely, if it does not, the system is 'below threshold', and the introduced infection cannot be maintained.

This discussion makes clear the underlying nature of the threshold relation for malaria. The relation can be written explicitly in terms of the number of mosquitoes, biting rates, and recovery and death rates of mosquitoes and people: as reviewed by Macdonald^{54,55} and Conway⁵⁰, this threshold formula has many implications for management.

The main fact, however, is that for simple models of the malaria transmission process there is always a unique stable state: for mosquito densities above threshold, there is some endemic level of infection; below threshold the level is zero. For many helminthic infections the situation is made more complicated by the parasites' having a sexual stage in the human host. Thus, for example, an adult female schistosome will produce eggs only if she has a mate, which may be unlikely if the mean worm load per person is less than, or of the order of, unity.

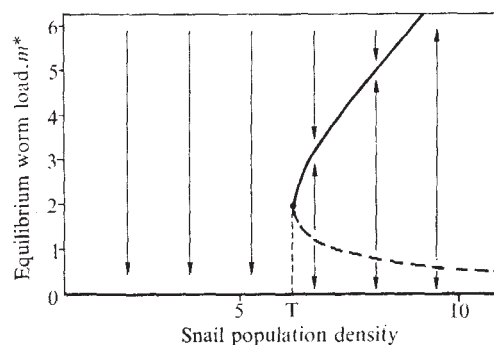


Fig. 8 For a simple model of the transmission dynamics of schistosomiasis, the equilibrium mean worm load per person, m^* , is shown as a function of the snail population density (or other relevant transmission factor). The threshold and breakpoint phenomena are as discussed in the text. (From Bradley & May⁵⁰.)

As first stressed by Macdonald^{29,55}, the result is that for schistosomiasis and other parasitic infections with a sexual stage in the primary host there is, as before, a threshold transmission value which depends on the population density of the snails or other intermediate vector, on the rate of egg production in the primary host, and on other transmission parameters. Below threshold, there is only one equilibrium state, namely no infection. Above threshold, there are two alternative equilibrium states: if the initial mean worm load per person, m , is sufficiently high, the infection cycle is maintained; but if m is initially too small, the number of mated females (which will scale as m^2) may be insufficient for the system to 'take off' and attain its endemic equilibrium level. This situation is illustrated in Fig. 8, which corresponds to simple models for schistosomiasis^{29,56-58}. If the snail density (or other relevant transmission factor) is below the threshold value T , the

equilibrium level of adult schistosomes is zero. Above threshold, the mean worm load per person moves either to the upper equilibrium curve, or to zero, depending on whether its initial value lies above or below the "breakpoint" line.

Figure 8 is for a closed system, with no immigration of infectious snails or people from outside. If a small amount of such immigration is included (I. Nasell, to be published) the lower equilibrium curve in Fig. 8 must shift up to some small, but finite, level of infection, and a figure similar to Fig. 6 is obtained (with m replacing N , and snail density or the like replacing S).

The existence of a breakpoint holds important implications for control of the system^{29,55-59}. For diseases such as malaria, where there is always a unique equilibrium state, the only control strategy is to reduce the mosquito numbers and/or the biting rate and other parameters in such a combination as to take the system below threshold. The system must, moreover, subsequently be kept below threshold. But infections whose dynamics are as described by Fig. 8 can in principle be eradicated by temporarily displacing the mean parasite load below the breakpoint, thus taking advantage of the two alternative equilibrium states. This control strategy has the advantage that the basic transmission parameters do not have to be modified to, and maintained at, sub-threshold values. (If the infection dynamics of snails operates on a fast time scale, and of humans on a slow time scale, the locus of the breakpoint depends only on the level of infection among humans; more generally, the breakpoint will depend on both snail and human infection levels⁵⁶⁻⁵⁸. It also depends on whether the adult parasites are distributed among humans in random or clumped fashion^{58,59}.)

Current estimates are that about 200 million people have schistosomiasis, and about 300 million suffer from the various filarial infections (which include 'river blindness' and elephantiasis). But empirical information on the overall transmission dynamics of schistosomiasis and filariasis is scant, so that theoretical notions about threshold and breakpoints remain untested in this context.

Conclusion

This review has drawn together a variety of mathematical models for ecological systems, each of which is dealt with individually in the scattered literature. The models are united by the common theme of possessing a regime of dynamical behaviour in which there are two alternative stable states, so that continuous variation in a control variable can produce discontinuous effects. Thus smooth changes in stocking rates can cause discontinuous changes in the grazed vegetation; continuous changes in harvesting rates can cause discontinuous collapse in fisheries; continuous changes in environmental parameters or foliage growth or predation rates can lead to discontinuous outbreaks of insect pests; continuous changes in snail or dipteran population densities can cause discontinuous appearance or disappearance of helminthic infections.

These models can often be tied to empirical data, whence they yield specific insights into the management of the system. (Some of these insights can be recast in the language of catastrophe theory, but this is usually *post hoc* window-dressing. It is too often forgotten that catastrophe theory is, strictly speaking, a local theory; we want global descriptions of the dynamics.)

The review has been largely confined to the dynamical behaviour of a single population, albeit as a function of other populations of resources and predators. As the number of interacting dynamical variables, and of equations, increases things get more complicated. Consider, for example, a predator-prey system where the predator's dynamics depends on the prey population according to the backward-bending curve of Fig. 6 (N versus S), and where the prey's dynamics in turn depends on the predator population according to Fig. 3 (V versus H). These two curves—one for the predator equilibrium, the other for the prey equilibrium—are in principle capable of intersecting at 9 points, corresponding to no fewer than four alternative stable states (separated by four saddle points and one central hilltop), each with its own domain of attraction. As the dimensionality of the system increases to encompass more and more species, the dynamical

landscape can begin to look like the surface of the moon, and any detailed comparison with field or laboratory data becomes very difficult.

A different and additional complication is that systems with discrete generations are described by difference equations, which can exhibit kaleidoscopic dynamics. For such systems, of which Fig. 5 is an example, increasingly severe nonlinearities can make the dynamical behaviour range from a stable point, through a bifurcating hierarchy of stable cycles, into a regime which is in many ways indistinguishable from random noise⁶⁰⁻⁶². Similarly, patterns of stable points or cycles giving way to chaos can be found in systems described by differential equations with time lags, or even in simple first-order ordinary differential equations once 3 or more species are interacting^{60,62,63}.

These manifold complications can lead one to take a gloomy view of the possibility of making predictions about multi-species systems^{60,62}. The one kindly light amidst this encircling gloom is that many complex communities are, arguably, made up mainly of loosely coupled lower-order systems^{17,64,65}, to which the simple models reviewed here are pertinent.

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articles

Hypervelocity cratering and impact magnetisation of basalt

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Suitably modified explosive shaped charges have been used to produce small hypervelocity projectiles ($v \sim 15 \text{ km s}^{-1}$) to study e.m. effects associated with high-velocity cratering of basalts, thus simulating in a terrestrial environment some features of meteoritic impact on the lunar surface. The experiments have shown that plasma is formed during impact and that an external field can be impressed in the basalt.

In an experiment to test whether meteoritic impact can magnetise lunar rocks (either by creating and impressing a transient magnetic field or by impressing and implanting a pre-existing field pattern), we made observations of magnetic phenomena associated with the cratering due to the impact of macroscopic ($m \sim 0.1\text{--}1 \text{ g}$) hypervelocity projectiles ($v \sim 15 \text{ km s}^{-1}$) on basalt blocks. For these experiments the 'meteoroid' consisted of the tip of the jet from a shaped charge and the target of a basalt block similar in composition to some lunar basalts. These tests were conducted in evacuated vessels, both with and without external magnetic fields either parallel or perpendicular to the basalt surface, and observations show that (1) a transient magnetic field followed by a short burst of electromagnetic oscillations is created near the impact region, and (2) it is possible to impress an externally applied magnetic field into the basalt.

Measurements of the magnetic field of lunar rocks have given values ranging between 10^{-2} and 1.2 Oe (refs 1, 2) for inducing fields necessary to account for the observed natural remanent magnetisation. Recent indirect observations from the Apollo 17 sub-satellite³ have confirmed the wide range of magnetic field intensities and orientations recorded by magnetometers flown on board previous Apollo missions.

Theoretical models based on lunar dynamo⁴ and fossil field theories^{5,6} have been invoked to explain the observations. Suggestions have also been made that there is evidence concerning the existence of a magnetic core⁷. Theories which can account, however, for both magnetic field amplification and/or local magnetisation mechanisms can explain more readily the wide range of observed values and are more amenable to direct experimental verification. Of these theoretical models, some assume the magnetisation to be associated with meteoritic impact⁸⁻¹⁰, while cometary impact has also been considered as a mechanism for impressing and magnifying an external magnetic field¹¹. Finally, during discussions with Professor K. Anderson, Space Science Laboratory, University of California, Berkeley in 1975 we considered the possibility that the plasma created during a hypervelocity impact could support transient electric

fields and associated electric currents, as observed in laser 'impact' experiments¹²⁻¹⁴. For hypervelocity impact, the residual magnetic field would be the remanent of an induced field, due to both the shock remanent magnetisation (SRM, or piezoremanence) and to the thermal remanent magnetisation (TRM) associated with the heating effects of the impact. The basic system of currents and inducing magnetic fields which can arise in laser 'impact' experiments, and can probably be present in hypervelocity impact experiments, are discussed in refs 15, 16 and illustrated in ref. 17 (see also for bibliography).

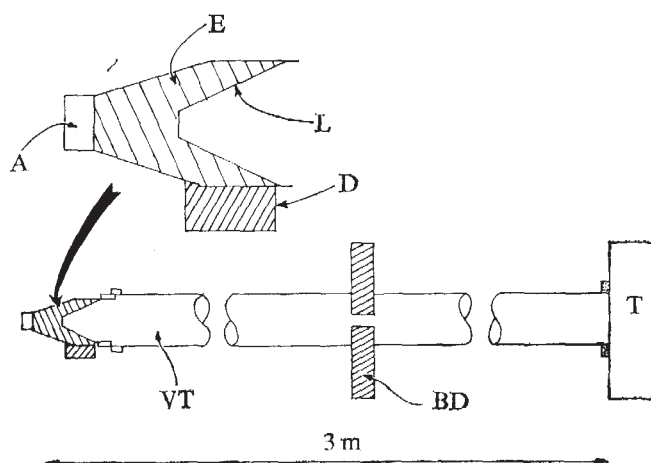
The only experimental technique we know of which has been used so far for the purpose of simulating shock compression associated with high-velocity impact is that of the explosively driven flying plate technique^{18,19} and interesting results have been achieved by this method²⁰. It differs, however, from the technique used here on a major point, since it does not actually simulate very closely the impact of a relatively small object on a large surface.

We describe here results from some experiments designed to simulate meteoritic impact, the 'meteoroid' consisting of the tip of the jet from a shaped charge, the target being a block of basalt with composition reasonably similar to that of some lunar basalt.

Experimental technique

Central to the experiments described below is the use of shaped charges to produce a high velocity jet of material, of which the

Fig. 1 Experimental set-up. L, Aluminium liner; D, deflector (TNT); E, explosive shaped charge (Octol); A, detonator; VT, vacuum tube; BD, blast deflector; T, target (basalt block).



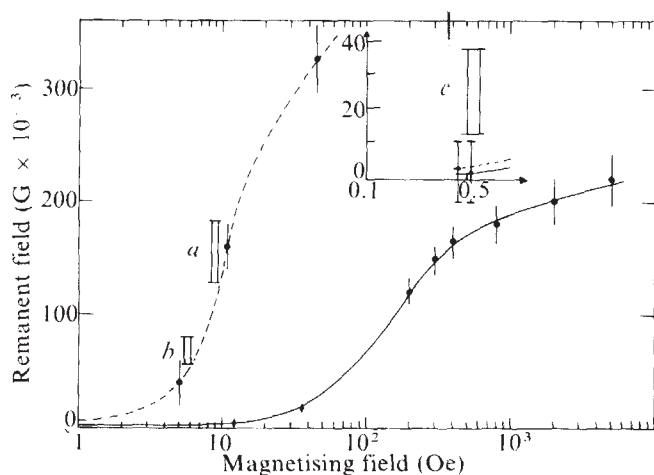


Fig. 2 IRM field (continuous curve) and TRM field (broken curve) for basalt samples used in the experiment; *a*, SARM for a 10 G field perpendicular to the target surface; *b*, SARM for a 6 G field parallel to the target surface; *c*, SARM for the geomagnetic field parallel to the target surface.

tip only is allowed to impact on the basalt target; the remaining, and slower, portion of the jet is deflected by means of a subsequent explosion. As is well known, a metal lined shaped charge consists of a cylinder of explosive having a high detonation velocity (v_d), the cylinder having a metal lined conical cavity at one end. When detonated at the other end, the (plane) detonation wave implodes the cone and parts of it are ejected with a velocity larger than the detonation velocity (Monroe effect). The maximum velocity v_m depends on the opening angle α of the cone and the ratio of the mass of material that lags behind (the 'slug') is also a function of α . This ratio can be improved by truncating the cone at, say, about one tenth of its height, measured from the apex. The velocity gradient along the jet causes it to stretch and break up into individual pellets. We are interested in the fastest fragments of the jet, hereafter referred to as the 'tip'. If the density ρ_l of the liner is close to that of the explosive ρ_e , the shock impedance of the liner is well matched to the implosion wave, thus minimising surface reflection and maximising the amount of liner material in the tip. Provided the experiments are conducted in an evacuated system, the temperature of the jet is not expected to exceed a few hundred degrees Celsius, because of the cooling associated with the adiabatic expansion which it undergoes during flight.

In our experiments the liner was made of HE30 aluminium

alloy, which has a relatively low density ($\rho_e = 2.73 \text{ g cm}^{-3}$), is easily machinable and non-toxic. The explosive was Octol ($v_d \sim 8.4 \text{ km s}^{-1}$, $\rho_e \sim 1.8 \text{ g cm}^{-3}$). The angle α was 45° .

The experiment

The experimental set-up is illustrated schematically in Fig. 1. The system was evacuated by a rotary pump to $\sim 10^{-2}$ torr. On ignition of the explosive at A, the propagation of the detonation causes: (1) the explosive ejection of the tip of the jet, and (2) the shock ignition of the TNT deflector.

If the TNT deflector is properly placed, only the tip proceeds unperturbed while the remaining solid and gaseous debris is swept sideways. The best positioning of the deflector was obtained by trials against plates which recorded the impact. It was found that the debris could be deflected through $\sim 30^\circ$, and that only a small tip would reach the target.

The velocity of the tip was deduced by timing the flight over a set distance (1.5 m), the passage being recorded by the rupture of current carrying foils. Maximum values of v_m of $\sim 15 \text{ km s}^{-1}$ were recorded.

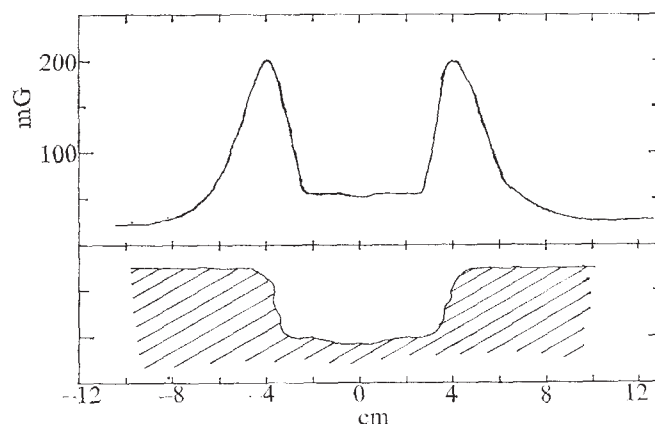


Fig. 3 A crater profile in a basalt block (shaded area). The field due to the SARM was measured 1.5 cm above the target surface, the magnetising field was $\sim 10 \text{ G}$ perpendicular to the target.

We believe that the differences in magnetic structure between lunar and terrestrial basalts do not invalidate the aims of our experiments. We have, however, attempted to select basalt which minimises these differences. This was found at a quarry at Rowley Regis near Birmingham, which supplied us with approximately 30 t of selected blocks. The composition of the samples is given in Table 1.

Experimental results

Magnetic mapping of the surface of the basalt blocks before and after impact was carried out using both a Hall probe and a flux-gate magnetometer.

Before impact the field at the surface of the basalt blocks was found to be of the order of $10 \pm 10 \text{ mG}$.

The magnetic properties of the basalt have been measured using a large number of cores, up to 2.5 cm diameter, extracted from various blocks. Figure 2 shows the contribution to the measured field due to the isothermal remanent magnetisation at room temperature ('IRM', continuous curve) and to the thermal remanent magnetisation ('TRM', broken curve) obtained after heating the samples to about 800°C and letting them cool inside a solenoid. The largest contribution to the error comes from the spread of values obtained from different samples. The size of the cores was chosen so as to make the

Table 1 Composition of some Basalt samples from the quarry at Rowley Regis

	%
SiO ₂	50.17
Fe ₂ O ₃	4.61
Al ₂ O ₃	18.75
CaO	7.33
MnO	0.16
MgO	5.41
K, Na oxide	4.93
FeO	5.79
Others	2.86

The blocks were cut, faced and inspected for homogeneity and absence of microfractures. The selected blocks were individually encased in alumina cement inside a reinforced dural frame, $45 \text{ cm} \times 45 \text{ cm} \times 25 \text{ cm}$, to prevent rupturing during test. The experiments were carried out in a reinforced concrete bunker at one of the proving grounds of the Raufoss Ammunition Factory, near Oslo.

field measurements practically independent of the core size, thus enabling the measurements to be compared directly with those at the surface of the basalt blocks.

We emphasise that, because of the short duration of the impact process, it is not possible to differentiate reliably between thermal remanent magnetisation and piezoremanent effects, so, hereafter, we refer to the magnetisation associated with the impact as 'shock associated remanent magnetisation' (SARM).

Three main types of hypervelocity impact tests were performed, and we present data from one of each. (1) with a 10 Oe external magnetic field at right angles to the basalt surface; (2) with a 6 Oe field parallel to the basalt surface;

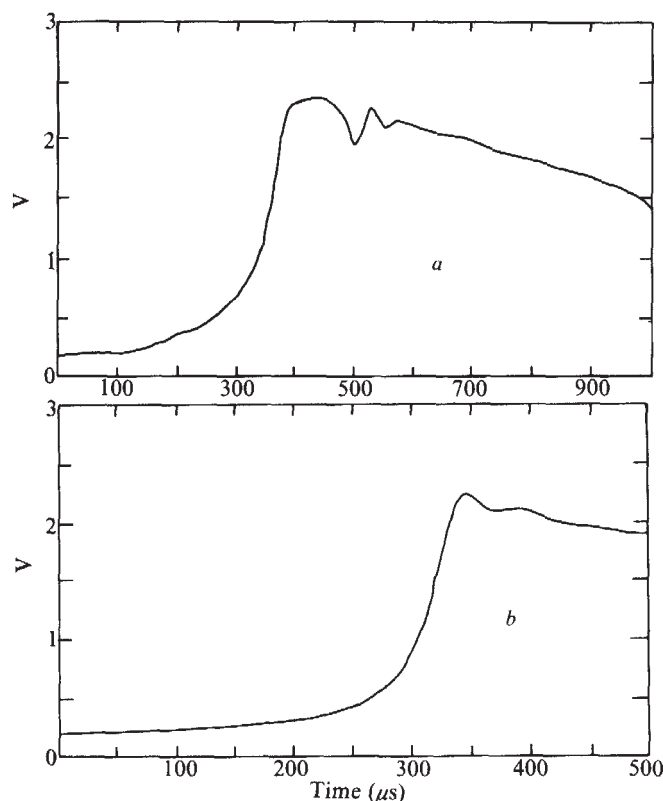


Fig. 4 Pickup signals from a coil surrounding the impact area. *a*, The vessel evacuated to 10^{-2} torr; *b*, in air. Note the different time scales.

(3) in the ambient magnetic field which, inside the bunker, was 0.5 G in the vertical direction.

The results of each of these tests are as follows:

Test type (a). Magnetic field measurements of the impact area yielded profiles such as that shown in Fig. 3. It can be seen that the largest SARM values are found in the neighbourhood of the field after impact is parallel to that of the externally applied field. The extreme values of the measured field due to SARM effects in the neighbourhood of the crater are shown in Fig. 2, as the error box marked 'a'.

Test type (b). In the region neighbouring the crater, relatively large magnetic fields were detected (approximately 100 mG). The maximum perturbations seem to be concentrated near the crater rim, along the fracture lines, that is, where the shock reached probably its highest value. The extreme values of the measured magnetic field for this test is also shown in Fig. 2, and is marked 'b'.

Test type (c). The results of the only impact experiment

conducted successfully in an evacuated vessel without an externally applied magnetic field, revealed magnetic perturbations at the basalt surface reaching up to ~ 40 mG near the crater (see inset in Fig. 2 marked 'c'). This value is no more than three to four times larger than the fluctuations recorded across the surface before impact. It may be that since the impact created a crater larger than was the case in the other tests, any more strongly magnetised material in the neighbourhood of the impact area may have been lost with the ejecta.

In some of the experiments mentioned above we recorded the electromagnetic noise generated on impact, using pickup coils surrounding the glass tube near the basalt surface and a 20-kHz bandwidth instrumentation recorder. In all cases we observed a rapid (probably bandwidth limited) transient magnetic field during impact followed by a number of oscillations (Fig. 4*a*). This suggests the creation of plasma capable of sustaining azimuthal currents, and possibly, transient flute instabilities. If this interpretation is correct, the current can be estimated from the amplitude of the recorded signals, to be of the order of 10^{-1} A. A similar experiment performed using the same geometry but at atmospheric pressure showed a faster transient magnetic field but much less pronounced oscillations (Fig. 4*b*). The question of how much plasma is formed on impact, at what temperature, and the type of transient instabilities generated therein needs further investigation.

Conclusions

Our experiments indicate that: (1) plasma is created by hypervelocity impact of small projectiles on a basalt surface; (2) an external magnetic field is impressed during impact, the SARM value being of the order of, or slightly exceeding that, of the TRM of samples of the same basalt. However, when the external field is as low as the geomagnetic field, the observed SARM is substantially larger than the corresponding TRM. (3) By extrapolating this result to impacts occurring in a field free region, it would seem that a magnetic perturbation can be created by the impact and impressed in the basalt. This point, however, requires further detailed measurements in a field-free environment.

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Thermodynamic theory of size dependence of melting temperature in metals

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The way that small particles melt is a crucial element in the construction of a thermodynamic treatment of the relation between particle size and melting temperature. There are indications that melting is initiated at the surface and that the solid-liquid interface sweeps rapidly through the solid at the melting temperature. The formal and physical elements of the indicated nucleation and growth criterion for melting are discussed and the existence of upper and lower limits on the melting temperature is outlined. Theoretical predictions show satisfactory agreement with experimental observations.

THE effect of particle size on melting temperature has promoted explicit theoretical discussions since the early 1900s and extensive experimental investigations have been reported. The qualitative experimental features are fairly simple—a monotonic decrease in melting temperature with decreasing particle size^{2–16}, this variation seems to be more substantial in the lower end of the size spectrum than in the 'large' size regime^{5–13, 15, 16}.

Previous theories have considered the solid-liquid-vapour mutual equilibrium¹⁷ (actually a size-dependent triple point^{18–20}) as the chemical equilibrium condition for a solid sphere embedded in bulk liquid¹⁸ (this, however, is related to the classical supercooling problem rather than to the melting of small particles), chemical equilibrium between solid and liquid spheres of identical radius¹⁹. In a later theory the 'thermodynamic melting temperature' was defined as that of Pawlow¹⁷ (although this is not necessarily the actual transformation temperature)²¹. Several of these and other works^{4–10, 19} have considered solid-liquid contact to be a central point: the favoured liquid skin theory considers chemical equilibrium between a finite liquid layer and the surrounded solid at the melting temperature^{4, 5, 7, 9}. Unfortunately, no physical criterion has emerged by which to determine the liquid thickness; this quantity (assumed constant) and the solid-liquid interfacial tension are determined by fitting the theoretical expression to experimental results.

In view of these problems associated with previous treatments of the size-dependence of melting temperature we have reconsidered the problem, and we present a thermodynamic theory of the relation between particle size and melting temperature.

Size dependence of melting temperature

Unlike in bulk systems, in small particles the mode of melting is a crucial element for the construction of a thermodynamic theory of melting. The size-dependence of intensive parameters in these systems requires that the geometry and extent of both phases be determined at the melting temperature. Consequently, it is imperative to consider the question of the mechanism of melting in general, and of small particles in particular.

First, note the essential asymmetry between melting and freezing—substantial liquid supercoolings are normal, whereas there is no significant superheating in solids. There is evidence that this lack of superheating is a surface-related effect^{22–24}; additional support for the important role of the surface in initiating melting is provided by kinetic studies of superheating^{25, 26}, low-energy

electron diffraction (LEED) studies of surface melting and freezing of (Pb, Bi and Sn) single crystal surfaces²⁷ and neutron scattering experiments indicating the absence of any bulk phenomena associated with melting in Al to within 10^{-1} K of the melting temperature²⁸. Moreover, molecular dynamics simulations of melting in small clusters of (rare gas) atoms indicate quite unambiguously that the transition is initiated at the surface^{29–31}. Finally, the fact that liquid metals wet the parent solid, energetically favours the initiation of melting at the surface²³.

Evidently, at the melting temperature a liquid layer is formed on the surface and moves at a substantial rate into the solid. It is these nucleation and growth characteristics of the melting phenomenon that may be used to construct a thermodynamic theory of the size-dependence of melting temperature.

The (closed, constant total volume) system considered is an isolated one-component collection of non-contiguous metal spheres at a uniform temperature and individually in mechanical equilibrium, resting on a non-interacting substrate. There are no dissociated species, no evaporation occurs during melting and gravitational effects are assumed negligible. Additionally, the difference between surface stress and surface free energy per unit area³² is neglected.

It is useful to recognise that a size-dependent absolute lower limit on the melting temperature exists. Neglecting the small vapour free-energy change this temperature, T_{lb} , is defined by the condition that the Helmholtz free energy difference between the final, entirely liquid, and initial, entirely solid, particles vanishes. The Helmholtz potential is appropriate as there is a pressure discontinuity associated with the melting of small particles. In equation (1) the subscripts s and l refer to solid and liquid; σ , areal surface work, r , particle radius; v , atomic volume; T_0 , bulk melting temperature; and l_0 latent heat, whence neglecting all but first order terms

$$T_{lb} = T_0 \left\{ 1 - \frac{3}{l_0} \left[\frac{r_s \sigma_s}{r_s} - \frac{r_l \sigma_l}{r_l} \right] \right\} \quad (1)$$

Although T_{lb} has been discussed as the two-state melting temperature^{7, 9, 34} it cannot be the actual melting temperature unless the mechanism by which the final state is attained is irrelevant (that is, in the limit of vanishing particle size).

The melting mode, and consequently the actual melting temperature, involves the formation of a liquid layer and a criterion to determine its spontaneous growth to consume the solid. The derivation and formal analysis of the Helmholtz free energy difference ΔF_1 between a core of solid surrounded by a concentric liquid layer and an entirely solid particle is thus complemented by the development of a melting criterion; their combined use leads directly to a quantitative estimate of the relation between particle size and melting temperature and, finally, to a comparison with experimental results. For brevity and simplicity we reproduce the principal features of the results of this energetic analysis by considering (where appropriate) only first-order terms. The superscript prime denotes quantities of the combined (solid-liquid) system. Those of the entirely solid system have no superscript. Pressure, temperature, chemical potential, number of atoms, volume and area are respectively, P , T , μ , N , V , A . The double subscript sl denotes quantities of the solid-liquid interface. Matter conservation is assumed between the solid and liquid states. Formally, ΔF_1 is given by

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$$\Delta F_i = -P_s'V_s' + P_sV_s - P_l'V_l + (\mu_s' - \mu_s)N_s' + (\mu_s' - \mu_s)N_l' + \sigma_{sl}A_{sl} + \sigma_l A_l - \sigma_s A_s \quad (2)$$

Explicit integration of the relevant Gibbs–Duhem relations provides a lengthy and analytically intractable expression for ΔF_i (ref. 1). Fortunately there are two simplifying approximations which retain all essential physical insight of the problem and provide an expression amenable to formal qualitative investigation: the liquid and solid atomic volumes are taken to be identical ($v_s = v_l \equiv v$) and both of these phases are assumed incompressible. These approximations yield the following expression

$$\Delta F_i = \frac{l_0(T_0 - T)}{T_0} \times \frac{4\pi}{3} \frac{r^3 - (r-t)^3}{v} + \sigma_{sl}4\pi r(r-t)^2 + (\sigma_l - \sigma_s)4\pi r^2 \quad (3)$$

where r denotes the initial solid (and here, final liquid) radius and t the liquid thickness. This function may be shown to have a stationary value (σ_{sl} , σ_l and σ_s are here approximated as size-independent) with respect to t (or, here, N_l) at constant temperature, when

$$\frac{l_0}{T_0}(T_0 - T) = \frac{2\sigma_{sl}v}{(r-t)} \quad (4)$$

This is simply the condition that the solid core has the same chemical potential as the surrounding liquid layer. For $\sigma_{sl} < 0$, physical solutions of this relation demand that $t > r$ and $T > T_0$. The extremum of equation (4) is then a maximum

$$\frac{1}{8\pi} \left(\frac{\delta^2 \Delta F_i}{\delta t^2} \right) r = -\sigma_{sl} \quad (5)$$

and thus, equation (4) defines an unstable chemical equilibrium. An approximate upper limit, T_{ub} , on the melting temperature may be inferred from the condition $\delta \Delta F_i / \delta N_l|_T > 0$ for vanishing t

$$T_{ub} = T_0 \left(1 + \frac{2\sigma_{sl}v}{l_0 r} \right) \quad (6)$$

Below T_0 the transformation of solid to liquid is favoured by the surface term (due to the wetting condition) and retarded due to the volume (latent heat) term. This is the converse of the classical nucleation problem and is consistent with the surface-induced nature of melting³⁵.

If the non-wetting condition $\sigma_s > \sigma_l + \sigma_{sl}$ is obeyed, the possibility of superheating exists; the condition $T < T_0$ restores the latent heat as the driving force for melting. It seems that a theory of the size-dependence of solid superheating analogous to the melting theory is possible. This explanation and such a theory relate to Peppiat's experiments²².

The Helmholtz free energy difference, ΔF_i , required to form a liquid layer on and from the completely solid particle is a maximum at the critical liquid thickness, t_c , defined by equation (4). In nucleation theory the probability of attaining this critical fluctuation will determine the speed of movement of the solid–liquid interface into the solid. A formal relation between temperature, the critical Helmholtz energy fluctuation ΔF_c and a measure, J , of this rate of movement is given by

$$J = C \exp[-\Delta F_c/kT] \quad (7)$$

where the pre-exponential factor C is only mildly temperature dependent and is taken as constant³⁵ and equal to that calculated by Stowell³⁶. The critical melting fluctuation ΔF_c , is formally given here by equation (3) evaluated at the chemical potential equality condition of equation (4). The various dependences (calculated, estimated or measured) of quantities needed to determine correction terms to the first-order expression quoted here are discussed in detail elsewhere¹.

Comparison of theory with experiment

A comparison of the predictions of this theory with experimental results is given in Figs 1–3. Sn, In and Au were chosen because these

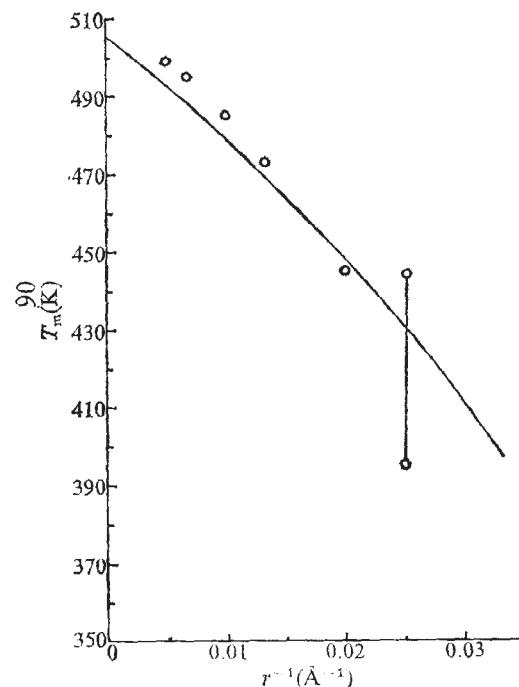


Fig. 1 Relation between melting temperature (T_m) and reciprocal particle radius (r^{-1}) for tin. —, Calculated; O, experimental. The latter were obtained directly or by interpolation from Wronski⁵ and are (except at $r^{-1} = 0.025 \text{ \AA}^{-1}$) midway between the experimental scatter. The scatter increases as particle size decreases and consequently is largest (and mostly unavoidable) at smallest r . At $r^{-1} = 0.025 \text{ \AA}^{-1}$ the apparent upper and lower values of T_m are illustrated. The solid curve represents the results of the calculation using equations (3, 4 and 7).

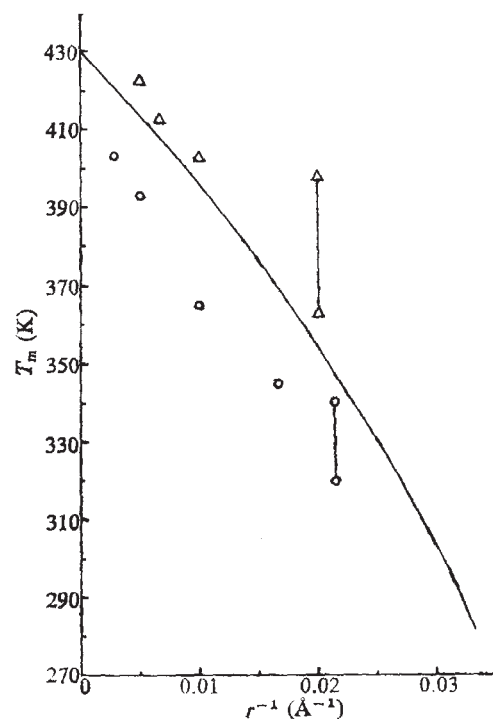


Fig. 2 Relation between melting temperature (T_m) and reciprocal particle radius (r^{-1}) for indium. —, Calculated; O, experimental (Pöcza, Barna and Barna¹³); Δ, (Berman and Curzon¹¹). The points are taken directly from Pöcza *et al.* and directly or by interpolation from Berman and Curzon. The scatter in the small size regime is illustrated for Berman and Curzon's data; the error bars reported by Pöcza *et al.* for small r are likewise given. The solid curve represents the results of the calculation using equations (3, 4 and 7).

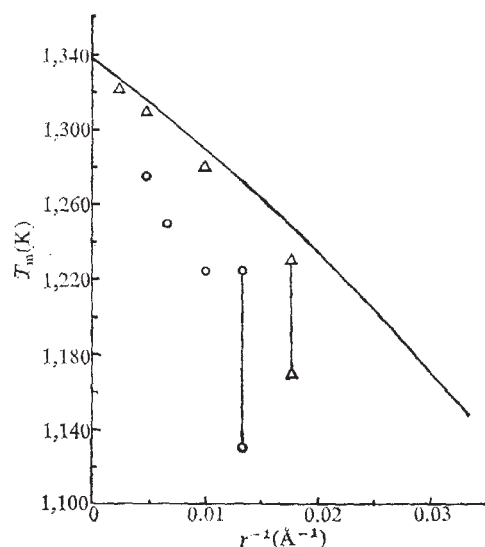


Fig. 3 Relation between melting temperature (T_m) and reciprocal particle radius (r^{-1}) for gold. —, Calculated; Δ , experimental (Sambles¹⁰); $\circ$, (Buffat and Borel¹⁶). Data obtained and treated as for Fig. 1.

were the only metals with both a sufficient number of surface parameters available (latent heats and heat capacities are relatively well documented) to allow theoretical calculations and a sufficient number of experimental results to allow a significant comparison between theory and experiment. The qualitative agreement is good. Further, the quantitative agreement is satisfactory in view of the approximations involved in the theory, the known lack of precision (and accuracy) in some of the quantities and their variation and the difficulty in obtaining accurate melting temperature and size measurements over such a large range. Actually, the only surface parameter we used which had any substantial experimental disagreement is σ_{sl} . We have used values consistent with the strong evidence that its direct determination from grain boundary grooving measurements³⁷ yields a significantly larger value than that inferred from supercooling measurements—the latter give a lower limit to σ_{sl} .

These calculations indicate that the critical liquid thickness (r_c) is not constant for each metal^{4,5,7,9} but decreases monotonically with decreasing particle size.

Conclusion

The thermodynamic theory of the size-dependence of melting temperature outlined here seems to provide a satisfactory and physically acceptable explanation of the phenomenon and is in reasonable agreement with experimental observations. Although other treatments have implied that solid-liquid interface movement is important in the transition^{4-9,15,19} (Tammann³⁸ was the first to suggest that melting is a surface phenomenon) none has sought to explore the surface nucleation and liquid layer growth characteristics of melting in order to provide a formal treatment of the size-dependence of melting temperature.

One expected and important feature of melting in small systems that this treatment predicts is the 'smearing out' of the transition as particle size decreases. This effect stems from the basic (nucleation and growth) characteristics of the melting phenomenon although its predicted extent is minimal; and the transition is predicted as extremely sharp down to very small particle size. ($< \sim 1$ K even at the small end of the size spectrum.)

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Corrected age of the Pliocene/Pleistocene boundary

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Calcareous plankton datum-events are referred to the Pliocene/Pleistocene boundary at Le Castella, the stratotype Calabrian at Santa Maria di Catanzaro, and to deep-sea sediments in six piston cores. The boundary is correlated by multiple overlapping criteria to a level equivalent to, or slightly younger than, the top of the Olduvai Event, giving a revised estimate of about 1.6 Myr for the age of the boundary. The Pliocene/Pleistocene boundary is thus coeval with the earliest of four major climatic deteriorations in the Pleistocene, reconciling palaeoclimatic concepts with the chronostratigraphical definition of the epoch.

ESTIMATES of the age of the Pliocene/Pleistocene boundary have varied from about 0.6 Myr¹ to > 4 Myr^{2,3}. The absence of reliable palaeomagnetic measurements on the stratotype of the Calabrian at Santa Maria di Catanzaro⁴ and the boundary-stratotype section at Le Castella^{5,6} in southern Italy has prevented direct correlation with the palaeomagnetic polarity 'scale'. Over the last decade biostratigraphical studies on the critical sections in southern Italy and also on palaeomagnetically-dated deep-sea cores have resulted in essentially two estimates for the age of the boundary: 1.6–1.8 Myr, by association with the Olduvai Event^{7–11} and 2.8 Myr, by association with the Kaena Event^{4,12–14}. Those who agree with the younger date have generally believed that the base of the Calabrian, as defined at Santa Maria di Catanzaro¹⁵, was stratigraphically equivalent to the 'marker-bed' at Le Castella¹⁶; these workers have

referred to the Le Castella 'marker-bed' section as the reference section for dating the age of the boundary. On the other hand, those who agree with the older date have consistently referred the boundary to a level near the physical base of the Catanzaro section. This level, the approximate transition from Pliocene clays to sandier beds above that indicate a different environment—the so-called Sandy Calabrian—is more than 75 m below the level of the prominent sandy calcarenite, Bed G-G' of Gignoux's defining study, which was the designated base of the Calabrian Stage originally and which is now placed at the base of the Calabrian stratotype^{17,18}.

We present here the results of a study of the calcareous plankton

(first appearance datum) of the members of the coccolithophore genus *Gephyrocapsa* in four of the palaeomagnetically-dated deep-sea cores (Fig. 1) and at Le Castella (Fig. 2). The minute size of the specimens (commonly 1.5–7 µm) and the subtlety of the criteria that distinguish various *gephyrocapsid* taxa, makes species differentiation (except the end members of the lineage, *G. aperta* and *G. oceanica*) under light microscope at best unreliable. Scanning electron microscopy, as used in this study, is essential (Fig. 4).

In core V12–18 *G. aperta*, the earliest (Fig. 1) and morphologically simplest form (Fig. 4a), appears at about 2.25 Myr. More evolved forms, such as *G. prototuxleyi* and *G. omega*, appear within the Olduvai Event in cores CH61–171 and V12–18,

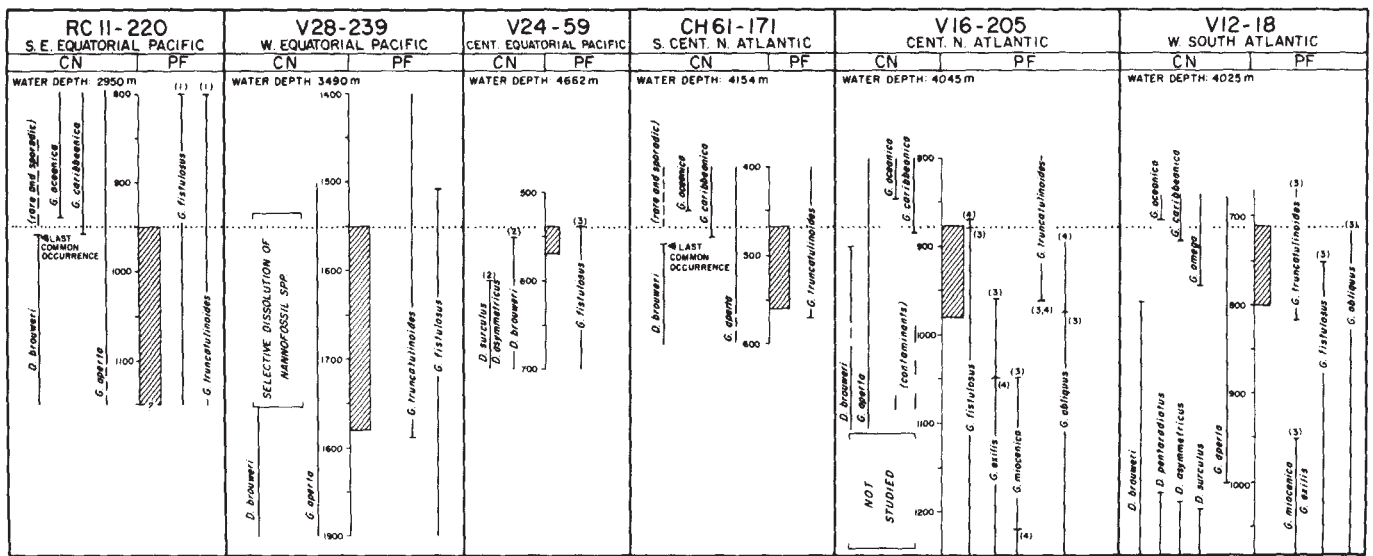


Fig. 1 Biostratigraphy of piston-core samples, showing ranges of taxa that are critical to the correlation of the Pliocene/Pleistocene boundary as related to normal-polarity Olduvai Event (hatched interval). Ranges are shown according to observations made in this study; previously published observations of range-limits in these cores are indicated by numbered points, as follows: (1), Bielak and Briskin⁴⁰; (2), Gartner⁴¹; (3), Saito *et al.*¹¹; (4), Briskin and Berggren³⁷. Location of the various cores is: RC11–220: 14° 49'S, 139° 58'W; V28–239: 03° 15'N, 159° 11'W; V24–59: 02° 34'N, 145° 32'W; CH61–171: 26° 41.5'N, 39° 23'W; V16–205: 15° 24'N, 43° 24'W; V12–18: 28° 41.7'S, 34° 29.6'W.

of six palaeomagnetically-dated deep-sea cores, and of the Calabrian sections of southern Italy. We have established a calcareous plankton biochronology in the deep sea cores that is applicable to these sections and which provides a more accurate means of estimating the age of the Pliocene/Pleistocene boundary than has previously been possible. We use the geomagnetic polarity scale of Cox¹⁹, consider the Gilsa and Olduvai events to be age equivalent²⁰, and assign ages of 1.61–1.79 Myr to the upper and lower limits of the Olduvai Event, respectively.

Material

We have examined, or reviewed literature on, calcareous plankton microfossils from six palaeomagnetically-dated deep-sea cores (Fig. 1). Planktonic foraminifera from core V16–205 were examined by W.A.B. for another study so that two sets of observations are shown for that core. Calcareous nannoplankton are selectively dissolved and fragmented over the interval of the Olduvai in V28–239, but the remaining biostratigraphical data on the cores is shown in Fig. 1. By way of comparison, calcareous nannoplankton and selected foraminifera have been examined in 60 samples from the stratigraphical section at Le Castella (Fig. 2) and samples from Santa Maria di Catanzaro (Fig. 3) which were collected by F. Barbieri in 1971.

Calcareous nannoplankton

The basic biochronological framework for correlating the Pliocene/Pleistocene boundary is provided by the sequential FAD

respectively. In four cores (CH61–171 and V16–205 from the South Central North Atlantic; V12–18 from the western South Atlantic; and RC11–220 from the South-East Equatorial Pacific) the upper boundary of the Olduvai is straddled by the FAD of *G. caribbeensis* (below) and *G. oceanica* (above). Mean ages of 1.62 Myr and 1.57 Myr respectively are assigned to these FAD events.

In the relatively long South Atlantic core V12–18, it has also been possible to assign ages to the LAD (last appearance datum) of the last four remaining discoasters of late Pliocene age. *Discoaster surculus*, *D. asymmetricus* and *D. pentaradiatus* disappear sequentially around 2.3 Myr, just before the FAD of *G. aperta* (Fig. 1). The terminal discoaster, *D. brouweri*, disappears within the Olduvai Event, but apparently slightly earlier (that is, near the base of the Olduvai, ~1.8 Myr) in the South Atlantic core V12–18 than in the Central North Atlantic (CH61–171, V16–205), or Equatorial Pacific (RC11–220, V24–59) where it disappears close to the top of the Olduvai Event, ~1.64 Myr. Specimens occur rarely and sporadically above this level in some cores. Considering the ease with which calcareous nannoplankton can be reworked into younger strata, the *D. brouweri* LAD as shown in cores CH61–171, V16–205, and RC11–220 may be slightly younger than the actual extinction level of this species. A three-rayed variety of this species becomes more numerous near the LAD in many cores.

The sequence of evolutionary events in the *Gephyrocapsa* lineage seen in the deep sea cores also occurs in the Le Castella section (Fig. 2). We have examined in detail 60 nannoplankton samples from this section under both light and scanning electron

microscopes. *Gephyrocapsa aperta* is found in sample CAT 3, some 105 m below the boundary marker bed, *G. protohuxleyi* is found in sample CAT 14, about 40 m below the marker-bed and morphologically typical *G. caribbeanica* (that is, comparable with the holotype) and *G. oceanica* are found in samples CAT 34 and CAT 36, ~30 and 25 m below the marker-bed, respectively. Throughout the Le Castella section, reworked discoasters of early and late Tertiary age are found. But, some 50 m below the marker-bed, *D. brouweri*, the terminal representative of the genus, decreases abruptly in abundance; approaching this level from below, it occurs relatively numerous and continuously and the three-rayed variety grows relatively more numerous. Above this level, however, it occurs relatively rarely and sporadically and about 15 m below the marker-bed it virtually disappears. This distribution pattern of *D. brouweri* has been observed previously²¹, and we consider the true LAD of *D. brouweri* to be ~50 m below the marker-bed.

Planktonic foraminifera

In the same samples, a series of planktonic foraminiferal datum levels are also associated with the early Matuyama Epoch, and the Olduvai Event in particular^{9, 11}.

In the subtropical South (V12-18) and North (V16-205) Atlantic the LAD's of *Globorotalia miocenica* and *Globorotalia exilis* fall within the interval of 2.2 to 2.0 Myr, slightly below the Olduvai Event. The FAD of *Globorotalia truncatulinoides* is associated with the base of the Olduvai Event, occurring some 10-20 cm below it in Equatorial Pacific core V28-239, and

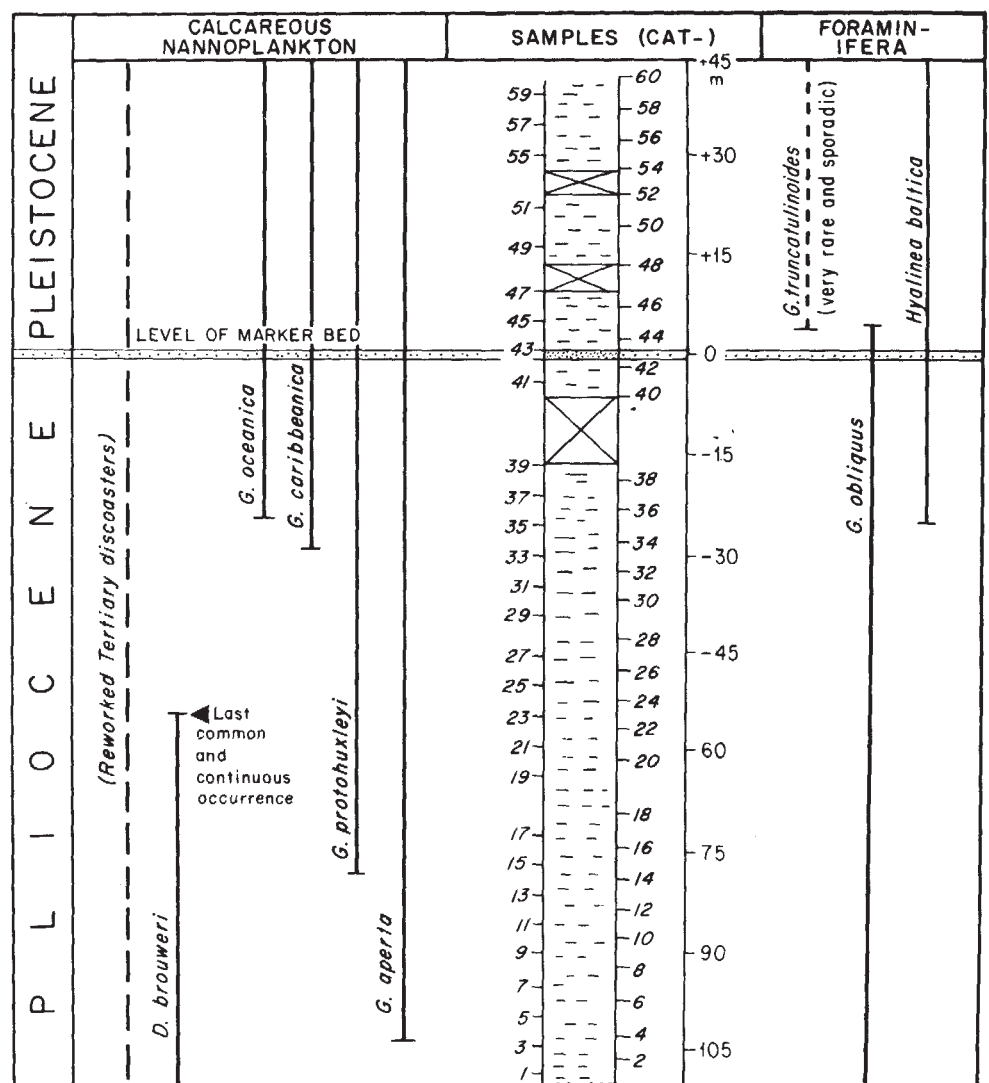
10-20 cm above it in subtropical North Atlantic cores V16-205 and CH61-171. The LAD of *Globigerinoides obliquus* is virtually coincidental with the top of the Olduvai Event in V16-205 and V12-18 (Fig. 1) and with the marker bed at Le Castella (Fig. 2). The LAD of *Globigerinoides fistulosus* also seems to be close to the top of the Olduvai Event (mid-part of Olduvai in V12-18; coincident with the top in V24-59 and slightly above it in V28-239). An anomalously young (~1.3 Myr) LAD of this species is noted in RC11-220.

The lowest recorded occurrence of *Globorotalia truncatulinoides* at Le Castella is ~2 m above the Pliocene/Pleistocene boundary (Fig. 2) and at Santa Maria di Catanzaro ~30-40 m below Bed G-G' (Fig. 3). Various other levels for this first occurrence have been cited^{1,6,16,22,23} but this unreliability seems to be due primarily to the extreme rarity of specimens.

Benthonic foraminifera

The calcareous benthonic foraminifer *Hyalinea baltica* is generally regarded as one of the primary biostratigraphical criteria for the base of the Pleistocene in the Mediterranean²². At Le Castella the FAD of this taxon is generally regarded as coincident with the lithostratigraphical marker-bed (between samples CAT 50 and 51) separating Pliocene and Pleistocene¹⁶. But, the range of *H. baltica* has been found²⁴ to extend at least 25 m below the marker-bed, at which level it constitutes 17% of the total benthonic fauna. At Santa Maria di Catanzaro *H. baltica* occurs near the base of the exposed section, at least 65 m below Bed G-G' (Fig. 4). If the FAD of this taxon was approximately simultaneous within the basin in

Fig. 2 Stratigraphical occurrence of calcareous plankton taxa and *Hyalinea baltica* at Le Castella (Calabria, Italy). Samples (CAT), collected by F. Barbieri in 1971, are the same as those studied by Rio²⁵.



Pliocene/Pleistocene^{16,26}. Correlation between the stratigraphic sections and the age of the boundary markers at Le Castella and Santa Maria di Catanzaro has caused much controversy. Orthodox opinion has been that the two marker-beds are stratigraphically equivalent^{16,22} and that the boundary is about 1.8 Myr^{10,11,27}. One strongly dissenting opinion^{12-14,28} has been that the true base of the Calabrian, and the Pliocene/Pleistocene boundary, is represented by the base of sandy Calabrian beds that extend more than 75 m stratigraphically below Bed G-G' at Santa Maria di Catanzaro, and that this level should be dated at about 2.8 Myr. In this view, the marker bed at Le Castella is about 1 Myr younger, and represents the base of the Emilian Stage. In contrast, another dissenting opinion²⁹⁻³¹ holds that the lowermost Calabrian—equivalent to the Le Castella section—is missing at Santa Maria di Catanzaro because the sandy Calabrian is transgressively unconformable on the clayey Pliocene beds, and as a consequence all of the Calabrian (*s.l.*) above this contact is younger than the Le Castella marker-bed. The contact has also been described as a fault¹⁸, but even so, Bed G-G' clearly seems to be younger than the marker-bed at Le Castella. This is because the entire 65 m of sandy Calabrian underlying Bed G-G' contains *Hyalinea baltica*²⁴ together with the various Pliocene/Pleistocene species of *Gephyrocapsa* noted above (Fig. 3; see also ref. 14) in an association that first appears at Le Castella only a short distance below the Pliocene/Pleistocene boundary (Fig. 2). The fact that *G. obliquus* has not been observed in the sandy Calabrian tends to confirm the correlation of this section to a level above the Le Castella marker-bed (see Fig. 5).

The presence of discoasters in the Catanzaro and Le Castella sections to levels well above the marker-beds has been cited^{14,16} as evidence that the Calabrian (and the Pliocene/Pleistocene boundary) is older than the Olduvai Event, but last occurrences of nannofossils are less reliable, due to reworking, than first occurrences as biochronological datums; in this particular case the argument that the sudden reduction in numbers of discoasters about 50 m below the Le Castella marker-bed represents the actual

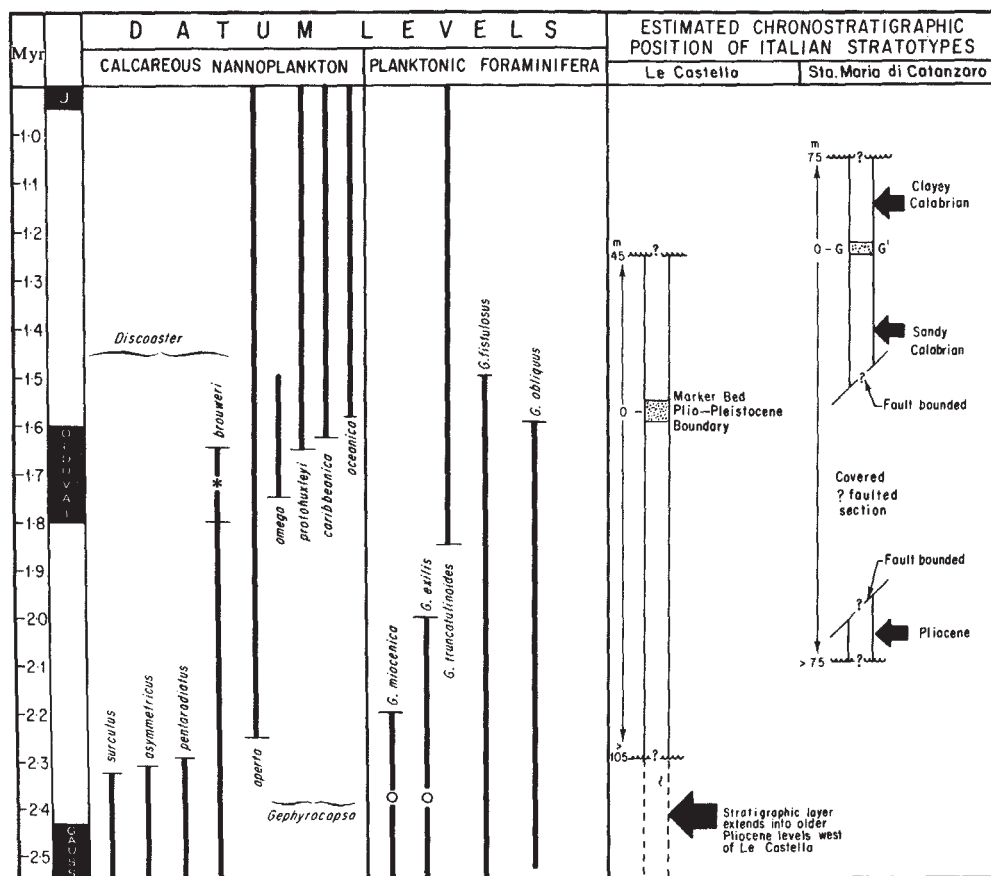
LAD agrees with the nannoplankton biostratigraphy in the deep-sea cores (Fig. 1). Thus, all of the discoasters collected in the Santa Maria di Catanzaro Calabrian (*s.l.*), both below and above Bed G-G' are probably reworked because they are stratigraphically above the FADs of *G. caribbeanica* and *G. oceanica* and the LAD of *G. obliquus*.

We seem now to be faced with a paradox. According to the criteria already cited, Bed G-G' at Santa Maria di Catanzaro (which denotes the base of the stratotype of the Calabrian Stage) is younger than the marker-bed at Le Castella (which denotes the Pliocene/Pleistocene boundary). If this is so, then the section of sandy Calabrian below Bed G-G' is both Pliocene because it is pre-Calabrian, and Lower Pleistocene because it is younger than the Pliocene/Pleistocene boundary. By accepting the view that there is only one definition of the Pliocene/Pleistocene boundary, that of Le Castella, we resolve the paradox and simultaneously demonstrate the value of the 'golden spike' in picking apart such conundrums.

Calabrian, Emilian and Sicilian

The stage-subdivisions of the Italian Lower Pleistocene are based partly on geomorphological and continental evidence, and partly on marine invertebrate biostratigraphy, because of the environmental and stratigraphic effects of glacio-eustatic changes³. Radiometric dating^{3,32,33} of strata assigned to Pleistocene stages, therefore, is not clearly applicable to the marine sequence, nor is the marine sequence well-correlated. To cope with this, Ruggieri and his colleagues³¹ proposed the use of invertebrate datum events—the FADs of *Arctica islandica*, *Hyalinea baltica*, and *Globorotalia truncatulinoides*—to identify the beginning (base) of the successively younger Calabrian, Emilian, and Sicilian Stages, respectively. In this proposed arrangement, the section at Santa Maria di Catanzaro from Bed G-G' upward is specifically correlated to the Sicilian Stage of Palermo³¹ because the occurrence of *A. islandica* in Bed G-G' is (rightly) held to be accidental,

Fig. 5 Pliocene/Pleistocene calcareous plankton biochronology in deep-sea cores and estimated chronostratigraphical position of about 1.8 Myr (*) in one of the cores studied (V12-18). The upper limit of this species, as shown here, may thus be somewhat younger than the actual extinction datum, due to reworking at the depositional interface. ○, Atlantic only.



but the occurrence of *Globorotalia truncatulinoides* close to this level is held to represent the true FAD of this species and thus the base of the Sicilian.

Aside from violating the principle that stratotypes, not fossils, define a stage, the proposal³¹ is untenable on the basis of biostratigraphy cited here. First, *H. baltica* ranges down through the sandy Calabrian at Catanzaro²³, and down to 25 m below the Pliocene/Pleistocene marker-bed at Le Castella²⁴, which would indicate that these strata belong to the Emilian, not the Calabrian Stage, and that there is, apparently, no Calabrian in Calabria at all. Second, the occurrences of *Globorotalia truncatulinoides* in the Pliocene/Pleistocene of Calabria are rare and unreliable, and the evidence from areas where it is more abundant indicate that its FAD precedes that of *G. caribbeanica* and *G. oceanica* (Fig. 5) and thus, one may presume, the FAD of *H. baltica* (see Fig. 2) In summary, to follow the proposal of Ruggieri and others³¹ in this region would first eliminate the Calabrian in its present form by invalidating its stratotype and burying it in the Sicilian Stage, and then would extend the base of the Sicilian (in theory) below that of the Emilian as well. These assumptions as to the relationships of the lower age limits of the proposed defining taxa are incorrect, or (in the case of the shallow-water mollusc *A. islandica*) not demonstrable, at least in the type Calabrian area.

Correlation of discoaster extinction level

We have observed that the extinction of discoasters at Le Castella, marked by a sudden drop in relative abundance of *D. brouweri* that signifies the transition from autochthonous to recycled status, is in close association with the sequential first appearance of various species of *Gephyrocapsa* (Fig. 2). The same relationship in the biostratigraphy of deep-sea cores that overlap the top of the Olduvai Event (Fig. 1) allows us to correlate the Pliocene/Pleistocene of Calabria to the deep-sea record (Fig. 5). Kaneps³⁴ has likewise observed that in equatorial-Pacific core RC12-66, the uppermost autochthonous occurrence of discoasters is within carbonate-minimum 17 near the top of the Olduvai Event, at about 1.65 Myr. But, at DSDP Site 132 in the Mediterranean this datum was found to be below Mediterranean carbonate-minimum M21, within the upper Gauss polarity-stage at about 2.6 Myr, and so it was suggested that the extinction datum of discoasters was correspondingly earlier in the Mediterranean basin than in the open ocean due to differential cooling of the Mediterranean. Our data suggest, on the other hand, that *D. brouweri*, the last of the discoasters, became extinct in the Mediterranean and in the tropical Pacific and Atlantic oceans almost simultaneously. The apparent diachroneity cited in DSDP Site 132³⁴ may be due to local effects, or to miscorrelation.

Palaeoclimatic history and the beginning of the Pleistocene

When the Pliocene/Pleistocene boundary was correlated to the beginning of the Olduvai Event according to the best data of a few years ago⁷⁻¹¹, oceanographers found that there were no identifiable major climate changes in the deep-sea record (the land record was simply too vague) that could be correlated to such a boundary. This seemed to justify a new approach to the boundary, implicit in the recommendation of the 1948 International Geological Congress³⁵ that the boundary be defined by the base of the Calabrian Stage in Italy—the beginning of the golden spike concept. Abandoning any reference to palaeoclimatology seemed healthy iconoclasm at the time, because of irritating and unreconcilable arguments that were beginning to arise out of radiometric dating on 'the earliest glacial event' ranging from 0.5 Myr to 3 Myr^{2,14} or more—evidently palaeoclimatic models had different attributes in different places.

The *Globorotalia truncatulinoides* datum has now proved to be a weak reed, and with the correlations described here we are on firmer, slightly different ground. It has long been supposed that the

change from Piacenzian clayey sediments to the markedly different lithology and colder-water fauna of the Calabrian was a reflection of Pleistocene climate replacing pre-glacial Pliocene^{1,6,27,28}, and the 1948 Commission referred to this in its recommendation³⁵. (For those unfamiliar with golden spikes, the reasons for establishing a physical reference point as a boundary definition become immaterial once the definition, or spike, is pounded in.) But, oceanographers, accustomed to the *truncatulinoides* connection, have habitually ignored or discounted the palaeoclimatic criterion as wishful thinking by workers bemused by over-dramatic concepts of the Ice Ages.

With the revised estimate of 1.6 Myr for the boundary, these conflicting concepts of the beginning of the Pleistocene seem to be reconciled. Synthesis of marine and nonmarine data for the Late Neogene had already shown that the first major glacial advance in North America, the Nebraskan, and correlative climate-induced effects elsewhere, should be dated close to 1.5 Myr³⁶, and that three others begin at approximately 0.9, 0.6, and 0.3 Myr respectively. More recently, quantitative factor-analysis and oxygen-isotope studies of microfossils in cores from the equatorial Pacific and tropical North Atlantic^{37,38} have been used to derive Pleistocene-palaeoclimatic curves. These show clearly-developed periodicities of 92,000 yr—as predicted long ago by Milankovitch according to computations of orbital precession and recently used, with varying success, in Late Pleistocene climatostratigraphy^{1,16,39}—superimposed on much broader and stronger climate cycles with a periodicity of about 500,000 yr. The earliest of four of these major cold-water, high-salinity, high-seasonality peaks seen in the North Atlantic core³⁷ centres at 1.5 Myr, just above the top of the Olduvai Event. This palaeo-oceanographic feature is clearly to be equated in age with the Nebraskan glaciation³⁶. From the data presented here, it can also be clearly equated with the Pliocene Pleistocene boundary as defined by the marker-bed at Le Castella.

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Cultured epithelial cells of cornea, conjunctiva and skin: absence of marked intrinsic divergence of their differentiated states

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Keratinocytes of three different epithelia grown in cell culture express a large number of differentiation markers with either no differences or relatively small differences, depending on the species. Much of the distinctive phenotype of these epithelia in vivo must be due to external modulation and relatively little, at least in the case of the human, to permanent intrinsic divergence during development.

THE corneal and conjunctival epithelia and the epidermis are stratified squamous epithelia. Cells in the basal layer of all three seem similar, but differentiation in the superficial layers is obviously different. The corneal and conjunctival epithelia do not possess the granular cell layer and anucleate stratum corneum typical of epidermis. Since all three epithelia rise embryologically from closely related precursors, it is not surprising that they should share some common properties; but as these epithelia later become very different, they might be thought to have diverged markedly and irreversibly during embryogenesis.

With proper fibroblast support, human epidermal cells (keratinocytes) can be grown serially in cell culture^{1,2}, where they show many of the differentiation markers characteristic of this cell type *in vivo*. Single cells give rise to colonies, each forming a stratified squamous epithelium containing multiplying and differentiating cells. The cells synthesise keratins (T.-T.S. and H.G. unpublished) and grow in size, eventually forming cornified (cross-linked) envelopes^{3,4}. The final stages of epidermal differentiation are promoted by placing the cells in suspension—a condition which does not permit the cells to grow; instead they become permeable to Trypan blue, their keratin filaments become detergent insoluble, they form cross-linked envelopes, and with the aid of serum plasminogen, their nuclei are digested⁵.

We describe here the behaviour of corneal and conjunctival epithelial cells when grown in the same conditions. They can multiply and carry out the same programme of differentiation as the epidermal cells, including some aspects not observed *in vivo*. All three cell types are clearly keratinocytes and, at least in the case of the human, their behaviour in common culture conditions is so nearly the same that we are not able to distinguish them. In the case of the rabbit, there are some persistent, though small, differences between the keratinocytes of different origin.

Fibroblast dependence and effect of EGF

In earlier studies of cultured corneal epithelial cells, fibroblast support was not provided^{6–8}, but epidermal keratinocytes have since been found to require the support of fibroblasts in order to form colonies¹. This support was usually provided in the form of lethally-irradiated 3T3 cells at a density of about $20,000\text{ cm}^{-2}$ inoculated together with or before the keratinocytes. In these conditions, the keratinocytes form expanding colonies, displacing the 3T3 cells from the vessel surface. The epidermal cells are serially cultivable and grow through many cell generations. In the human, epidermal cells do not transform into established lines and retain the diploid chromosome number¹.

This culture system was applied to epithelial cells of cornea and

conjunctiva. Human corneas were obtained from local eye banks and after the endothelial layer and Descemet's membrane were removed with jeweller's forceps, the remaining tissue (epithelium plus a small part of the stroma) was minced to approximately $1\text{--}2\text{ mm}^3$ and digested with 0.25% trypsin and 0.002% EDTA at 37°C for 30–45 min. The disaggregated single cells were then plated with irradiated 3T3 cells. Multiplication of human fibroblasts was inhibited by the 3T3 cells, but if necessary, human fibroblasts were selectively removed with 0.02% EDTA after the epithelial colonies grew to appreciable size^{1,3}.

Figure 1 shows subcultures containing corneal epithelial and epidermal colonies 11 d after inoculation of 5×10^4 cells together with irradiated 3T3 cells. The colonies stained red with Rhodanile Blue and were of similar size and appearance. In the absence of 3T3 cells, neither epithelial cell type formed colonies; as there were no fibroblast colonies either, the cultures did not contain appreciable numbers of living human fibroblasts.

In the presence of epidermal growth factor⁹ (EGF), the cells of large colonies of epidermal keratinocytes sustain their growth rate much better than in its absence². Corneal epithelial cells were similarly responsive, judging from the size of the colonies (Fig. 1). Such an effect of EGF is to be expected from its action on intact corneal epithelium¹⁰. Conjunctival epithelial cells were similar to corneal epithelial cells in their colony-forming properties and EGF responsiveness.

When examined under the phase microscope, the basal cells of colonies of human corneal epithelial cells (Fig. 2a) seemed similar to those of epidermal cells (Fig. 2b) in their shape, their mosaic-like arrangement and their ability to displace the 3T3 cells from the vessel surface. The stratified structure of the colonies, and the silver-staining properties of the superficial cells³ were also very similar.

Four strains of corneal epithelial cells, originating from donors aged between 25 and 60, and growing in the presence of EGF, were subcultured twice and grew through a total of about 30 cell generations. Although epidermal cells of newborn donors can grow through many more generations², the culture lifespan of corneal epithelial cells in the present experiments seems similar to that of epidermal cells of adult donors¹.

Synthesis of keratins and other cellular proteins

The keratins of bovine epidermal stratum corneum have been characterised electrophoretically^{11,12}. Those of cultured human epidermal cells and human stratum corneum have recently been found to be similar in solubility, electrophoretic behaviour, immunological reactivity and ability to assemble into filaments *in vitro* (T.T.S. and H.G., unpublished).

The proteins of cultured epidermal, corneal and conjunctival epithelial cells were compared by electrophoresis in polyacrylamide gels containing sodium dodecyl sulphate (SDS). Figure 3a (track 1) shows the total protein of human corneal epithelial cells extracted with 2% SDS and 1% β -mercaptoethanol. Identical patterns were obtained from epidermal and conjunctival cells. In addition to the large number of protein bands unrelated to keratins, there were present several intense bands of keratins with a molecular weight range from slightly greater than that of actin (42,000) to slightly greater than that of tubulin (55,000). The non-

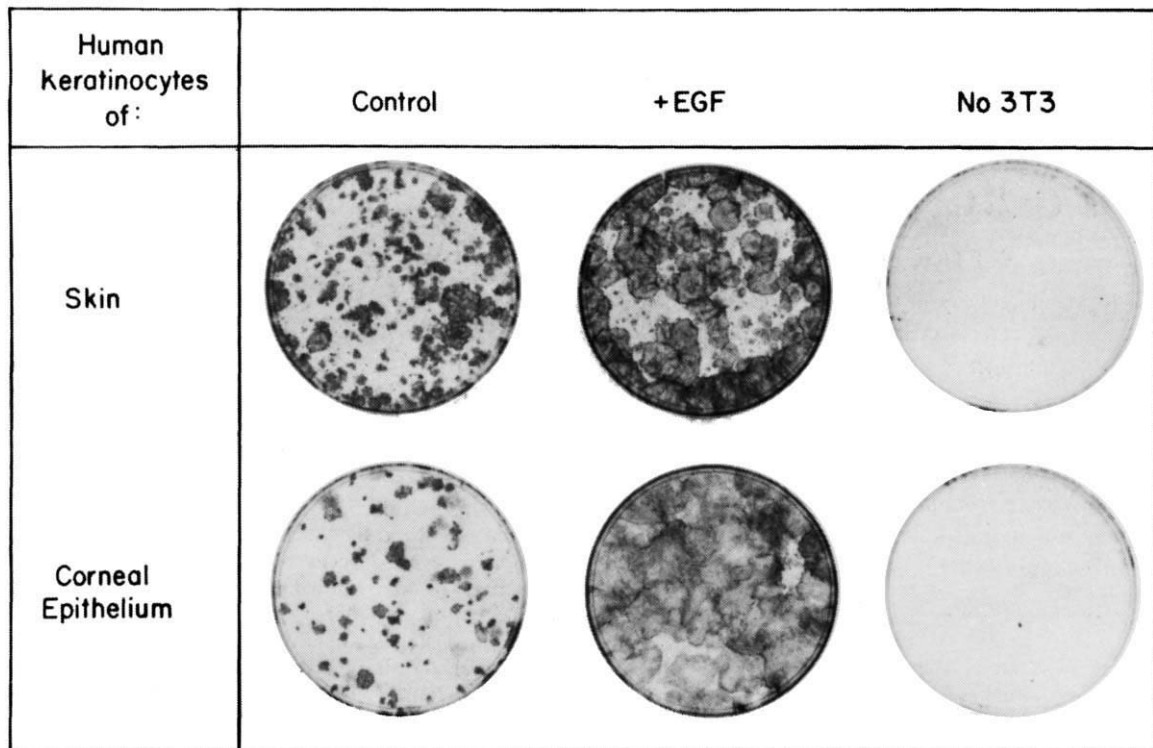
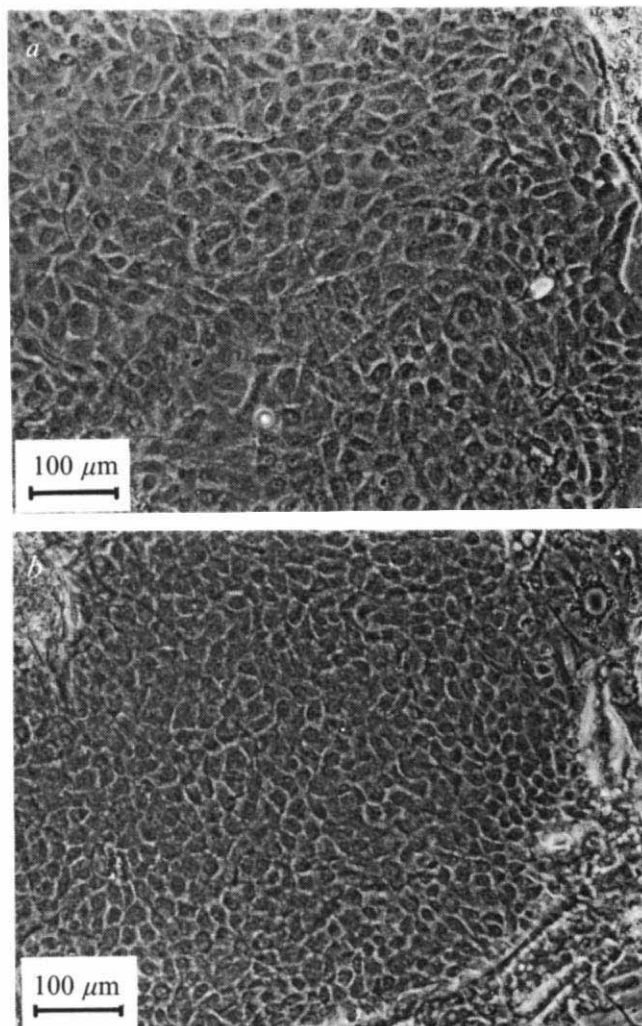


Fig. 1 Colony formation, fibroblast dependence and the effect of EGF. Human corneal epithelial cells (5×10^4 , strain B, donor aged 25 yr, secondary culture) and epidermal cells (5×10^4 , strain M, newborn donor culture) were plated in 60-mm dishes with or without 4×10^5 lethally-irradiated 3T3 cells. To some dishes, epidermal growth factor (EGF) was added to a final concentration of 15 ng ml^{-1} , starting on the third day after plating². After a total of 11 d, the cultures were fixed with 10% formalin and stained with Rhodanile Blue¹⁹.



keratin proteins can be removed selectively by extraction in dilute buffer, leaving substantially only the keratins to be extracted with SDS and reducing agent (Fig. 3a, tracks 2–5). Tracks 2, 3 and 4 show no consistent differences in the position or relative intensity of the keratin bands of corneal and conjunctival epithelial cells and of epidermal cells. No keratins were detected in fibroblast extracts (track 5).

In order to compare the keratins of the different cell types further, antiserum specific for epidermal keratins of human stratum corneum was prepared and tested in double diffusion experiments against an extract of the total protein of each cell type. It was found that the proteins of cultured epithelial cells of epidermis, cornea and conjunctiva reacted strongly with the antiserum and showed a precipitin band in common with one of the bands produced by the keratins of stratum corneum (Fig. 4). A second precipitin band produced by the keratins of stratum corneum was not present in any of the cultured cell types and may have been due to a 63,000-molecular weight protein known to be present in stratum corneum, but not in corneal epithelium or any cultured keratinocytes (unpublished). No visible precipitin band was produced by the proteins extracted from human fibroblasts.

Increase in cell size and formation of cross-linked envelope

During differentiation in culture, human epidermal cells increase their cell size and protein content³ as they do *in vivo*¹³. Figure 5a shows that, like epidermal cells, trypsin-disaggregated corneal epithelial cells adopted an approximately spherical shape and could be seen to vary greatly in size. The smallest cells, which are probably the basal cells³, had a diameter of about $12 \mu\text{m}$. The

Single colonies of corneal epithelial and epidermal cells. Primary colony of corneal epithelial cells of strain B (a) and secondary colony of epidermal cells of strain M (b) 11 d after inoculation. The cultures were fixed with buffered 10% formalin and photographed directly without staining. One margin of each colony appears in the right hand side of the pictures. Phase-contrast microscopy.

largest cells, probably the superficial cells of the epithelium³, exceeded 35 μm in diameter. Some of the large cells possessed cross-linked envelopes similar to those made by terminally differentiated epidermal keratinocytes. The presence of these envelopes was demonstrated by heating a trypsinised cell suspension in the presence of 5% SDS and 1% β -mercaptoethanol, a procedure which dissolves entirely cells without such envelopes but only the intracellular contents of cells with envelopes³ (Fig. 5b). The insolubility of the envelopes is due to proteins cross-linked by ϵ -(γ -glutamyl)-lysine bonds⁴. As in the case of epidermal cells, usually 5–10% of the cells in surface cultures of corneal epithelial cells possessed cross-linked envelopes.

Cross-linked envelopes were also found in human corneal epithelium *in vivo*. Samples were scraped from the surface of excised corneas with a knife, and treated directly with detergent and reducing agent. Microscopic examination showed that envelopes were abundant. These envelopes preserved the flattened shape of the cells *in vivo*, as they do even in cultures of epidermal

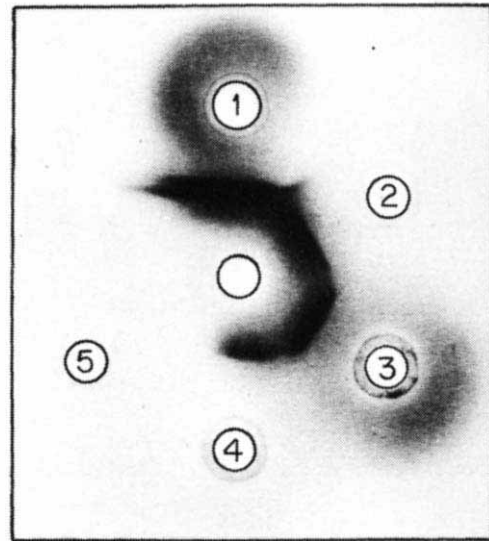


Fig. 4 Immunological cross-reactivity between keratins of human stratum corneum and proteins of cultured human epithelial cells. Stratum corneum was minced and pre-extracted with 8 M urea to remove non-keratin proteins. The keratins were then dissolved in 8 M urea containing 10 mM dithiothreitol. Rabbits were immunised at multiple sites with a total of 20 mg protein over a period of 2 months. Antiserum thus obtained was placed in the centre well of an Ouchterlony plate and tested against 80–120 μg of total cell protein of different cell types extracted with a solution containing 1% SDS, 10 mM dithiothreitol and 10 mM Tris-HCl (pH 7.4). 1, Keratins of stratum corneum (30 μg); 2, extract of cultured epidermal keratinocytes; 3, extract of cultured corneal epithelial cells; 4, extract of cultured conjunctival epithelial cells; 5, extract of cultured human dermal fibroblasts. The double diffusion test was performed in an agar plate containing 0.1% SDS, 0.5% Triton X-100, 0.01% Thimerosal, in addition to phosphate buffer and 1% Agarose, basically according to Yen *et al.*²⁰.

keratinocytes when the cells are treated with the reducing agent and detergent without previous trypsinisation³.

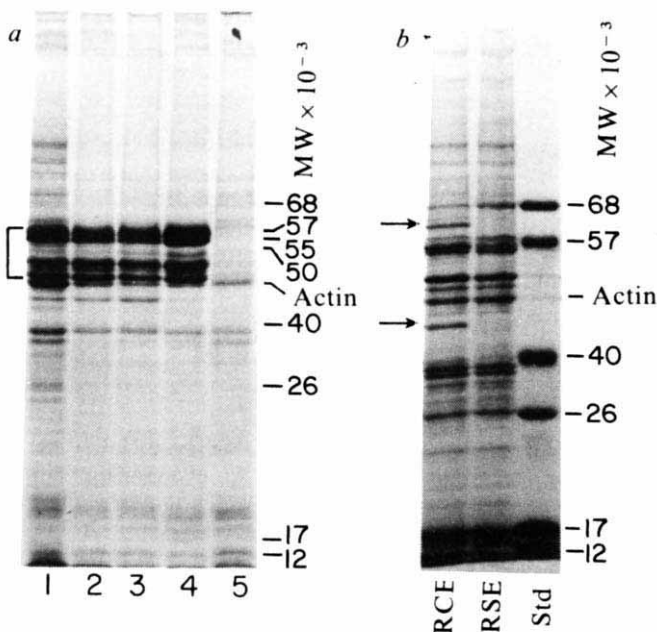
Terminal differentiation of suspended cells

When human epidermal cells are suspended as single cells in medium stabilised with methylcellulose (methocel), they develop disulphide-stabilised keratin filaments and become insoluble in SDS solutions. These conditions also greatly favour the formation of cross-linked envelopes. Eventually, the cell nuclei are destroyed⁵.

By all three criteria, human corneal epithelial cells were found to behave similarly. Within a few days in suspension, all the cells became insoluble in detergent, and about 50% developed cross-linked envelopes (Fig. 6). About 80% of the cell nuclei were destroyed within 9 d.

Properties of rabbit keratinocytes in culture

In order to compare the same cell types from another species, experiments were carried out on epidermal and corneal epithelial cells of the rabbit (newborn to 1 yr old). Both formed stratified squamous epithelial colonies in surface culture and developed detergent-insoluble keratin filaments and cross-linked envelopes in suspension. Both cultured cell types were shown to possess desmosomes and tonofilaments on electron microscopic examination, and both contained keratins demonstrable by electrophoresis (Fig. 3b). It is clear that, as in the human, both cell types are keratinocytes; the two cell types of the rabbit could, however, be distinguished in culture. For example, although the corneal epithelial cells formed cross-linked envelopes in methocel suspension, they did so with lower frequency than epidermal cells and did not form envelopes at all in surface culture. This is perhaps related to the fact that, unlike that of the human, the corneal epithelium of the rabbit *in vivo* does not possess cells with cross-linked envelopes. Rabbit corneal epithelial cells grew poorly on subculture in comparison with the epidermal cells. Finally, the



The proteins of cultured epithelial cells of cornea, conjunctiva and epidermis. *a*, Human, 3T3 cells were removed from nearly confluent epithelial cultures with isotonic buffer containing EDTA (refs 1, 3). The epithelial cells were collected in a small volume of the same buffer with the aid of a rubber policeman. Half of the cells were extracted directly with a solution of 2% SDS and 1% β -mercaptoethanol at 100 °C for 5 min. The extract was analysed by 12.5% disc polyacrylamide gel electrophoresis in the presence of SDS. The other half of the cells were first extracted with several changes of 100 vol 20 mM Tris-HCl (pH 7.4) and the insoluble proteins were then dissolved in detergent and β -mercaptoethanol as before and subjected to electrophoresis: protein (50–100 μg) was applied to each slot. The direction of electrophoresis was from top to bottom. Numbers on the right hand side of the gel denote molecular weights of standard proteins including human serum albumin (68,000), tubulin (57,000 and 55,000), the heavy chain of human gammaglobulin (50,000), and actin (42,000). The bracket at the left hand side of the gel indicates the mobility range of the keratin proteins. Samples in different tracks are: 1, total proteins of cultured human corneal epithelial cells; 2, insoluble proteins of the same cultured corneal epithelial cells; 3, insoluble proteins of cultured conjunctival epithelial cells; 4, insoluble proteins of cultured epidermal keratinocytes; 5, insoluble proteins of cultured human foreskin fibroblasts. Any apparent difference in band intensity between the extracts of the three epithelial cell types was not reproducible. *b*, Rabbit, 12-d-old primary cultures of corneal epithelial cells and epidermal cells derived from the same 1-yr-old animal were collected. Total cellular protein was extracted and analysed as for the human. Arrows show two non-keratin proteins present only in the corneal epithelial cells. The keratin bands, which lie between actin and molecular weight 57,000, differ slightly in mobility from those of the human and may be less abundant. RCE, Rabbit corneal epithelial cells; RSE, rabbit epidermal cells; Std, molecular weight standards.

cultured rabbit corneal epithelial cells contained two water-soluble non-keratin proteins not present in epidermal keratinocytes (Fig. 3b).

External modulation or intrinsic divergence of differentiated state

By the criteria already described, the cultured cells of all three epithelia are keratinocytes (to be distinguished from fibroblasts of corneal stroma, sometimes referred to as keratocytes). In the human cell cultures we have been unable to distinguish the three cell types by any criterion. The same differentiation markers can be expressed in culture by epidermal and corneal epithelial cells of the rabbit, although the culture phenotypes of the two cell types are not identical. These results suggest that an important part of the differences between these epithelia *in vivo* results from differences in the local environment in which the cell type is found.

One obvious local difference is related to the adhesiveness of the superficial cells of the epithelium. A dry-surfaced epithelium (skin) invariably possesses an anucleate stratum corneum, whereas wet-surfaced epithelia frequently do not. Whether an anucleate cell layer is present or not may simply be a matter of the adhesiveness of the superficial cells, which in turn could be affected by the degree of hydration. If adhesiveness is low, detachment may occur before differentiation is complete. This occurs in surface cultures of epidermal keratinocytes; nuclear destruction is completed, for the most part, after detachment of the squames⁵, rather than before, as in intact skin. Similarly, we have shown here that nuclear destruction takes place in corneal keratinocytes suspended in methocel much as it does in epidermal keratinocytes, although corneal epithelium does not normally possess a stratum corneum.

A second and perhaps more important difference is related to the nature of the cells located beneath the epithelium. Instructive effects of the underlying connective tissue are known to specify keratinocyte behaviour in adults¹⁴ as well as in embryonic life¹⁵. In our cultures of the various epithelia, the fibroblast population consists mainly of (irradiated) 3T3 cells. For the reasons mentioned, fibroblasts of the type associated with the epithelium *in vivo* were virtually absent from the cultures studied. The very similar phenotype exhibited by the cultured keratinocytes might be explained by either absence of specific fibroblast instruction or the presence of common instruction provided by the 3T3 cells. The possibility exists that any one of these keratinocyte types, especially in the human, placed in the *in vivo* site of one of the other, even after birth, would conform in phenotype to the other keratinocyte type and generate the site-specific epithelium^{14,16}.

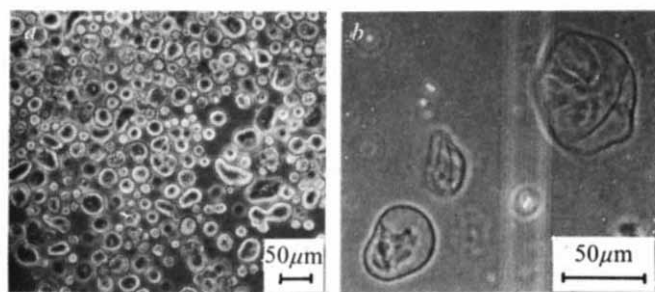


Fig. 5 Cell enlargement and formation of cross-linked envelopes by cultured human corneal epithelial cells. *a*, Heterogeneity of cell size in the growing colonies. 3T3 cells and any contaminating living fibroblasts were removed selectively from a nearly confluent 17-d primary culture with EDTA. The remaining corneal epithelial cells were trypsinised and an aliquot of the single-cell suspension examined in a haemocytometer chamber under the phase microscope. Note the presence of cells of variable diameter from 12 μm to $> 35 \mu\text{m}$. *b*, Cross-linked envelopes formed in surface culture. To a trypsinised single-cell suspension of cultured human corneal epithelial cells, SDS and β -mercaptoethanol were added to final concentrations of 5% and 1%, respectively and the solution was heated to 100 $^{\circ}\text{C}$ for 3 min. The photograph taken under phase microscopy shows several of the large envelopes remaining. All small cells are dissolved.

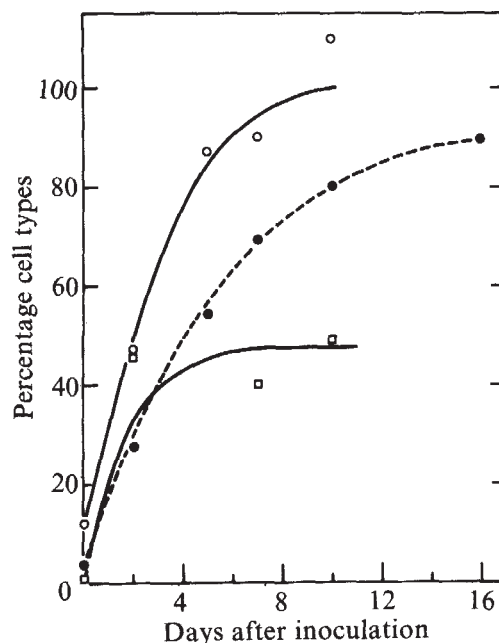


Fig. 6 Terminal differentiation of human corneal epithelial cells in methocel-stabilised suspension. Thirteen-day tertiary cultures of human corneal epithelial cells grown in the presence of EGF were trypsinised and resuspended at 2×10^5 cells ml^{-1} in medium containing 20% foetal calf serum and 1.2% methylcellulose. Aliquots were removed at intervals, diluted in isotonic buffer and the cells centrifuged. Cells insoluble in detergent (○) alone possess disulphide-stabilised keratin filaments. Cross-linked envelopes (□) were scored by insolubility in ionic detergent plus reducing agent. Cells were also deposited on filters, fixed and stained with haematoxylin and eosin, and the proportion of cells possessing nuclei was determined⁵. ●, Anucleate cells.

3T3 cells are known to be fibroblasts¹⁷, but it is not known from what organ they originated, as the line was evolved from mixed mouse embryo cell cultures¹⁸. This may not be important insofar as ability to support proliferation is concerned, since human diploid fibroblasts of non-dermal as well as of dermal origin support the growth of epidermal keratinocytes^{1,19}. The experiments described here also show that the ability of 3T3 cells to support keratinocyte multiplication is not specific for the type of keratinocyte. Although the fibroblast products necessary for the support of keratinocyte growth have not yet been identified, they may be different from those involved in instructive effects.

Whatever the special conditions that may modulate their differentiated state *in vivo*, the three cell types can, in the human, show a common phenotype in culture in which all the properties examined, including the differentiated ones, are expressed equally. In the case of the rabbit, although all the differentiated properties can also be expressed in culture by corneal and epidermal cells, the differences between the two cell types grown in identical culture conditions show that conversion to a common phenotype is not complete. Thus in this species, the intrinsic differences may make a significant contribution to the phenotypic differences between the two epithelia *in vivo*.

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letters to nature

Rapid fluctuations of radio flux and polarisation in quasar 3C273

QUASAR 3C273, one of the brightest and most studied quasars, is variable over a wide wavelength range. The changes of its luminosity may occur on a timescale of a few years or of several days in both the radio¹ and optical^{2,3} range. There are variations of linear and circular polarisation in the radio domain⁴ and sometimes the optical emission is circularly polarised⁵. The data from our simultaneous radio and optical observations show rapid variations of radio flux and circular polarisation at $\lambda = 1.35$ cm, and of optical linear polarisation of 3C273 within one day or several hours. These variations are possibly more rapid than those previously reported.

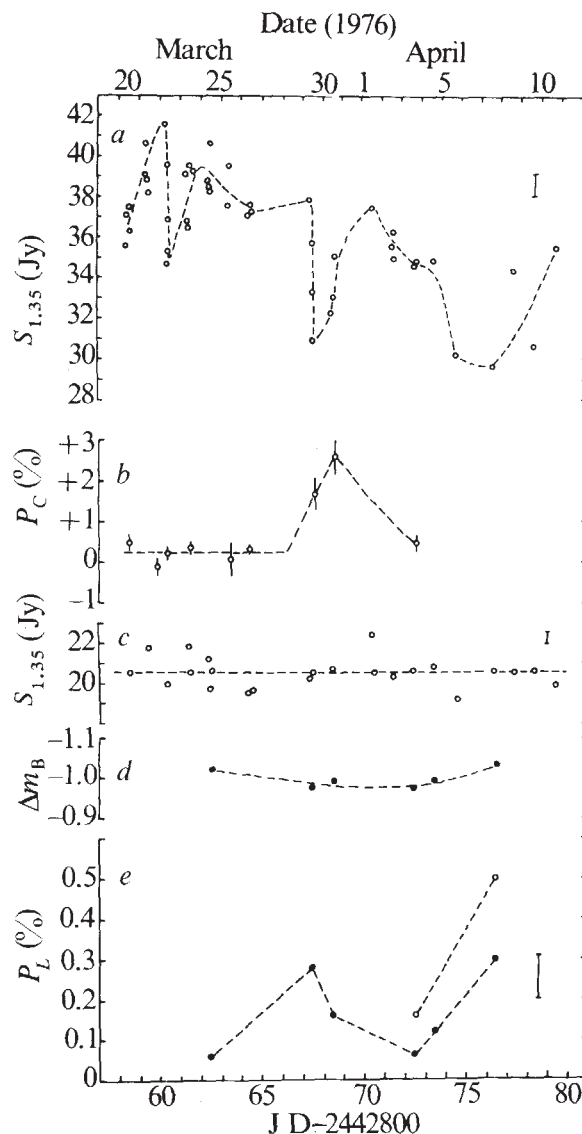
Total flux I and intensity V of circular polarised emission at 1.35 cm of the quasar 3C273 were measured during March–April 1976 using the 22-m radio telescope of the Crimean Astrophysical Observatory. The angular resolution of the radio telescope was $2.5' \times 2.6'$ and the sensitivity was about 0.7 Jy with a time constant of 1 s using a switched receiver with a maser as a high-frequency pre-amplifier. Beam switching in the azimuth plane was used to reduce the influence of fluctuations of atmospheric radio emission. The quarter-plate analyser was placed behind the central circular feed. The main and reference beams had orthogonal polarisation and measurements of flux I were made by an 'on-off' method similar to that described in ref. 6. A minicomputer operating on-line processed the output signals of the receiver and checked its amplification⁷. Flux density was measured relative to the standard sources Saturn (brightness temperature 128 ± 5 K) and the thermal source DR21 (flux density 19.5 ± 0.6 Jy). The water line at 1.35 cm lies in the received band, so the atmospheric attenuation may be variable. Therefore, to eliminate the influence of the atmospheric extinction variations the standard sources and the control source 3C274 were measured just before or after 3C273 and at about the same zenith distances. The corrections for extinction in this case did not exceed a few per cent. The r.m.s. error of the total flux determined with the aid of 3C274 does not exceed 2.5%. Zero level for parameter V was found by observation of DR21 which is expected to have zero circular polarisation; it showed no fluctuations in excess of the r.m.s. errors (its mean value found here is $-0.35 \pm 0.07\%$ where minus corresponds to left-handed polarisation).

Concurrently with radio observations of 3C273 optical observations were carried out with a polarimeter⁸ at the Cassegrain focus of the 2.6-m Crimean reflecting telescope. The optical intensity and the linear polarisation were recorded in B and V spectral bands with $\lambda_{\text{eff}} = 430$ and 540 nm respectively. The optical flux was measured with

respect to the comparison star 50'' to the West of 3C273 and polarisation was calibrated with the aid of standard stars⁹.

The results of radio and optical measurements are shown in Fig. 1. They sometimes show rapid intensity fluctuations

Fig. 1 *a*, Variations of the radio flux of 3C273 at 1.35 cm; *b*, the degree of circular polarisation 3C273 at 1.35 cm; *c*, the radio flux of control source 3C274; *d*, the optical brightness 3C273 at the B band in magnitudes; *e*, the degree of linear polarisation of optical radiation in the B (●) and V (○) bands. Vertical bars show the value of r.m.s. error.



of radio emission of 3C273 in a period of one day (22 and 29 March, 5 and 7 April) whereas the optical brightness remains nearly constant. The total (peak-to-peak) amplitude of relative variations of the radio flux from 3C273 is 33%, twice as large as that of the control source 3C274. The ratio of corresponding dispersions is 11.3 and is statistically significant at the $<0.5\%$ level. On the two days 22 and 29 March, variations of the radio flux of 3C273 reaching 20% were observed over about 4 h, although the intensities of 3C274 measured at the beginning and at the end of these runs did not differ significantly. The rapid decrease of radio flux on 29 March was accompanied by an increase in the degree of circular radio polarisation p_c from the average value of $0.24 \pm 0.07\%$ to $2.1 \pm 0.2\%$ and by the appearance of the optical linear polarisation beyond the 3σ range. Another such fluctuation of optical linear polarisation took place near 7 April when the radio flux was also at a minimum. The planes of polarisation on these two occasions were quite different (see Fig. 2).

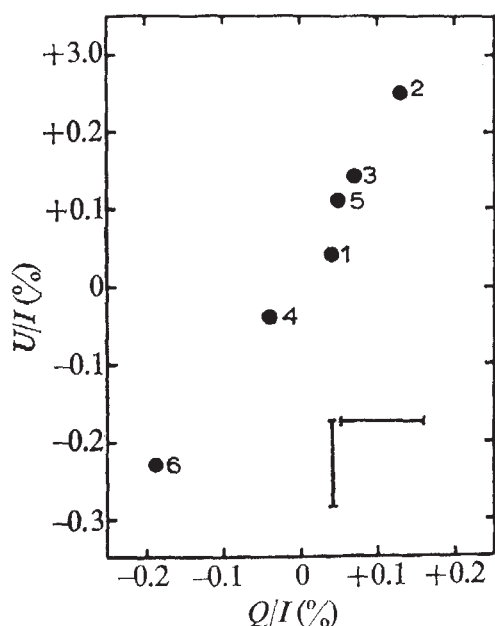


Fig. 2 Diagram of normalised Stokes parameters Q/I and U/I for optical linear polarisation of 3C273 at the B band. 1, 24 March; 2, 29 March; 3, 30 March; 4, 3 April; 5, 4 April; 6, 7 April 1976. Bars show the r.m.s. errors of each parameter.

A similar phenomenon, but with a slower decrease of the flux of 3C273 by 20%, was observed earlier at a wavelength of 3.5 cm on 23–27 May, 1969 (ref. 9). There is also a report about the decrease of the flux density at 7.2 cm of another source OJ287 of the same amount (20%) over about 2 h (ref. 10). It is remarkable that in OJ287 there is a similar correspondence between daily variations of its flux at 1.35 cm and that of optical linear polarisation as in the case of 3C273 mentioned here: optical emission is more polarised near the minimum radio flux^{11,12}.

The observed fluctuation of linear (in optical range) and circular (in radio range) polarisation could eventually be connected with possible rapid changes of transversal and longitudinal components of magnetic field in the quasar 3C273 during the periods of strong diminution of radio flux. This also agrees with the earlier observations of temporary appearance of circular polarisation in the optical range reported in ref. 5 and of the variations of the linear and circular polarisation of this quasar at radio wavelengths⁴.

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New high energy γ -ray sources observed by COS B

LOCALISED γ -ray sources contribute to the overall galactic emission; some of these sources have been identified with known astronomical objects^{1,2}, while several unidentified γ -ray sources have also been reported^{3,4}. We describe here a search for γ -ray sources using data from the ESA γ -ray satellite COS B which revealed 10 new unidentified sources. These sources seem to be galactic with typical γ -ray luminosities above 100 MeV in excess of 10^{35} erg s⁻¹.

The COS B satellite was launched in August 1975, and most of its (typically 1-month) observations have been devoted to a sensitive survey of the galactic plane. The characteristics of the instrument and the important features of the mission are described elsewhere^{5,6}. The data from four of the observation periods, for which manual analysis procedures⁶ are complete, have been analysed for sources of γ rays of energy > 100 MeV, using a method which yields an angular resolution of about 2.5° (FWHM). In each case the search has been limited to a region 40° wide in galactic longitude and 30° wide in latitude centred on the pointing direction. The method of analysis has been described in detail elsewhere⁷.

From the γ -ray intensity distributions the statistically most significant enhancements⁷ have been taken together with PSR0833–45, to compile the catalogue of γ -ray sources shown in Table 2. The nomenclature CG $n+m$ is introduced for a γ -ray source detected by COS B at longitude n° and latitude m° (truncated values). Table 2 lists the galactic coordinates, and the intensities above 100 MeV relative to the Crab (CG185–5). The indicated uncertainties in the relative intensities include systematic errors, reflecting possible changes in the instrument sensitivity, for which corrections are being derived. The intensity of CG185–5 above 100 MeV of $(3.5 \pm 1.0) \times 10^{-6}$ photon cm⁻² s⁻¹, is found to agree with other measurements⁸.

The latitude distribution of the detected sources, shown in Fig. 1, immediately indicates their galactic nature. The absence of sources between 7° and 15° is particularly significant because the visibility of sources increases with galactic latitude due to the

Table 1 Observations used in the search for localised sources

Pointing direction l°	b°	From	To
185	–6	17 August	17 September 1975
74	0	28 November	24 December 1975
322	0	23 February	24 March 1976
126	1	24 June	25 July 1976

decrease in the background flux contributed by the galactic disk. The distribution suggests typical distances in excess of 1 kpc which, coupled with typical flux values of 10^{-9} erg cm $^{-2}$ s $^{-1}$, leads to individual luminosities in excess of 10^{35} erg s $^{-1}$.

The nature of this new class of astronomical objects is intriguing. The positional errors virtually exclude unique identification with a known object, and the finite angular resolution makes it impossible to distinguish between a compact object and an emission region of angular dimension up to 2° . Although our results may not be representative for the entire galaxy, the sources do contribute significantly to the overall γ -ray luminosity of the galaxy, in the anti-centre region even accounting for most of the emission⁹.

Table 2 γ -Ray sources detected by COS B

Source	$l^{\text{II}}(^{\circ})$	$b^{\text{II}}(^{\circ})$	Relative intensity	Remarks
CG64+0	64.5 ± 0.5	0.0 ± 1.0	$0.30^{+0.10}_{-0.05}$	
CG75+0	75.0 ± 1.0	0.0 ± 0.5	$0.45^{+0.15}_{-0.10}$	
CG78+1	78.5 ± 0.5	$+1.5 \pm 0.5$	$1.00^{+0.30}_{-0.20}$	
CG121+3	121.0 ± 1.0	$+3.5 \pm 1.0$	$0.20^{+0.10}_{-0.05}$	
CG135+1	135.5 ± 1.0	$+1.5 \pm 1.0$	$0.30^{+0.15}_{-0.10}$	
CG176-7	176.0 ± 1.0	-7.0 ± 1.0	0.50 ± 0.10	
CG185-5	185.3 ± 0.5	-5.6 ± 0.5	1.00	PSR0531+21 at $l^{\text{II}} = 184.6, b^{\text{II}} = -5.8$
CG189+1	189.0 ± 1.5	$+1.0 \pm 1.5$	0.40 ± 0.10	
CG195+4	195.9 ± 1.0	$+4.5 \pm 0.5$	0.90 ± 0.20	195+5 (SAS-2)
CG263-2	263.7 ± 0.3	-2.6 ± 0.3	3.30 ± 0.50	PSR0833-45 at $l^{\text{II}} = 263.6, b^{\text{II}} = -2.8$
CG312-1	312.0 ± 1.0	-1.5 ± 1.0	0.45 ± 0.15	
CG327-0	327.5 ± 1.0	-0.5 ± 1.0	0.35 ± 0.15	
CG333+0	333.5 ± 1.0	0.0 ± 1.0	0.70 ± 0.20	

The suggestion by Black and Fazio¹⁰, that interstellar clouds could be γ -ray sources when bombarded by cosmic-ray protons, has been investigated. If the density of cosmic rays is the same beyond 1 kpc as is measured locally, masses in excess of $10^6 M_{\odot}$ would be required to account for the observed source intensities. No such massive clouds have been observed near the locations of the reported γ -ray sources.

A search for possible X-ray counterparts of the γ -ray sources has also been made. The weak sources 4U0241+61 and 4U1416-62 (ref. 11) are within the error boxes of CG135+1 and CG312-1 respectively. The soft source Cyg X-7, associated with the supernova remnant DR4 (ref. 12) falls within the error box of CG78+1. In addition, hard X-ray emission from the region of CG135+1 has been reported¹³. Such positional coincidences, however, provide only a weak argument for correlation in view of the high density of X-ray sources and the size of the γ -ray error boxes. In any case the absence of strong X-ray counterparts implies that the new γ -ray sources have substantially higher luminosities above 100 MeV than in the 2-6-keV band. It is noted that CG78+1 is not positionally coincidental with Cyg X-3, for which strong periodic γ -ray emission at an earlier epoch has been reported¹. Moreover, a search for the 4.8-h periodicity in the γ -ray emission from this region has yielded a negative result¹⁴.

Of the 149 known radio pulsars only PSR0531+21 and PSR0833-45 are confirmed γ -ray emitters. Of the remainder only one (PSR0611+22) falls within the error box of one of the COS B sources (CG189+1), for which a search for pulsating γ -ray emission has been inconclusive¹⁵.

Future steps towards a better understanding of the results presented here should include: (1) sensitive searches for radio pulsars and weak X-ray sources near the positions of the unidentified sources and (2) searches for timing signatures from candidate counterparts; in this context it is noted that CG195+4 shows periodic variations^{1,16}. This search is far from complete, as only one-third of the galactic plane has been effectively studied. Further analysis of available COS B data will provide a galactic distribution of γ -ray sources which will permit a comparison with other galactic populations.

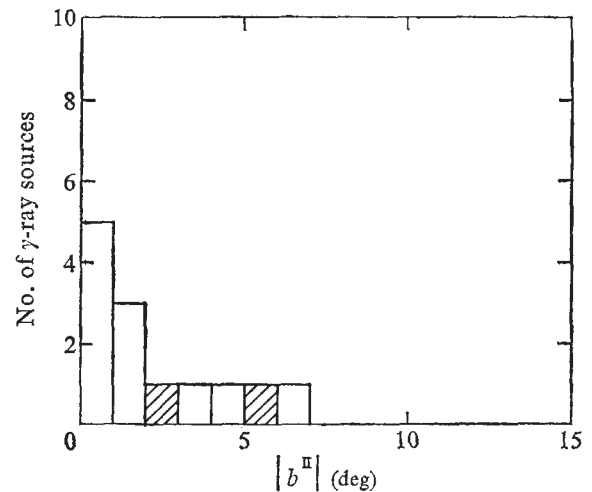


Fig. 1 Latitude distribution of γ -ray sources. The shaded area indicates the identified γ -ray sources PSR0833-45 and PSR0531+21.

The COS B mission is planned to continue until the end of 1978. Although much of the observation time is to be devoted to prolonged observations of the galactic plane from $l^{\text{II}} = 340^\circ$ to $l^{\text{II}} = 90^\circ$, special observations to check on the consistency of suggested counterparts for already detected sources may be considered.

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Positions of galactic X-ray sources Cir X-1, TrA X-1 and 3U1626-67

THE positions of three celestial X-ray sources were measured with the rotating modulation collimators (RMC) on SAS-3 during a survey of the galactic plane¹ and are precise to $\leq 25''$ (ref. 2). One of these sources, Cir X-1 (ref. 3), is a highly variable X-ray source often compared with the black-hole candidate, Cyg X-1. Another, TrA X-1 = A1524-61, is a 1974 X-ray nova⁴, and the third, 3U1626-67 (ref. 5), is a 7.68-s X-ray pulsar⁶. The results reported here support recently proposed optical and radio identifications⁷⁻⁹ of these three sources. In two cases (TrA X-1 and 3U1626-67), they have been instrumental in bringing about the proposed identifications. Cir X-1 exhibits a binary periodicity¹⁰ of 16.6 d, extreme aperiodic variability on timescales of > 0.1 s (refs 11, 12) and 1-3 s (ref. 13), and flaring^{14,15} with time constants of a few ms. This variability and the absence of a shorter (spin) period^{13,15} have led to suggestions^{11,13,15} that this object may be similar to Cyg X-1. The absence of a compelling optical or radio counterpart, however, has prevented the further exploration of this hypothesis.

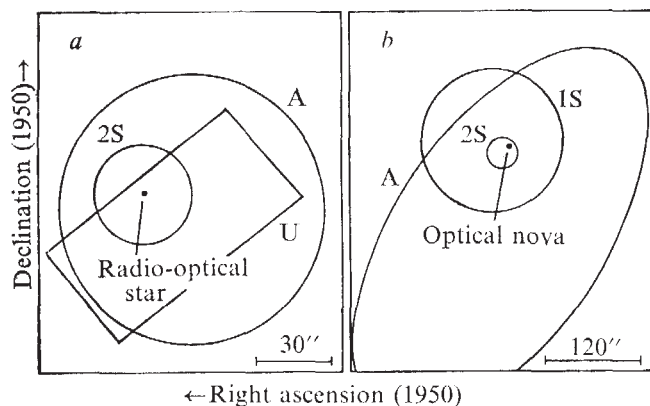


Fig. 1 X-ray positions and error regions (90% confidence) for *a*, Cir X-1 and *b*, TrA X-1. The Ariel V, A, (refs 19, 8); Uhuru, U, (ref. 11) and SAS-3, 1S, (ref. 21) results precede the present work, 2S. The locations of the optical and radio counterparts⁷⁻⁸ are indicated. (Finding charts are presented in refs 7 and 8).

A faint ($R \sim 16-17$, $B - R \sim 6$) optical star with strong H α emission¹⁶ and a flaring radio source^{17,18} have been proposed as counterparts to Cir X-1 on the basis of Uhuru^{5,11} and Ariel V¹⁹ X-ray positions. The radio source has been shown⁷ to lie within $2''$ of the H α star and the radio flares have been observed to occur 0.5-3.0 d after several of the periodic (16.6-d) X-ray eclipses. This establishes its identification and its present

2S1627-673

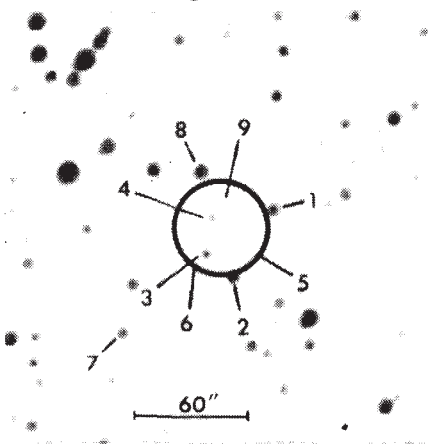


Fig. 2 Finding charts for the source 3U1626-67 - 2S1627-673. The optical candidate is star 4 (ref. 9).

position (Fig. 1, Table 1) removes any remaining doubt about the association of the X-ray source with the radio-optical star.

TrA X-1 was a classic X-ray nova first observed on 12 November 1974 with the Ariel V satellite⁴. It became as bright as the Crab Nebula on ~ 3 December 1974 and decayed with a time constant of ~ 2 months²⁰. SAS-3 was launched in May 1975, and the source was detected with the RMC system in June 1975. Preliminary analysis of the SAS-3 data yielded a position²¹ accurate to $1.5'$. This together with a revised Ariel V position has made possible the recent discovery⁸ of an optical nova that was observed at $B = 17.5$ on a plate taken 15 December 1974 with the Anglo-Australian Telescope. The star subsequently faded with a time constant of ~ 117 d. Earlier limits of $B > 19.5$ and $R > 19$ were obtained 20 and 5 months respectively before the X-ray maximum⁸. The present refined SAS-3 position, accurate to $20''$, lies $14''$ from the position of the optical nova (Fig. 1).

The source 3U1626-67 recently was found⁶ to be a 7.68-s pulsar with no evidence for a binary Doppler shift ($P_{orb} \lesssim 0.5$ d or $P_{orb} \gtrsim 35$ d). It has a hard X-ray spectrum and is variable⁶ by a factor 3 on timescales of 100-300 s. Shortly after the discovery of the periodicity, the position of the X-ray source was measured with SAS-3. The celestial area defined by the measurement is smaller by a factor of 50 than that previously obtained⁵ with Uhuru. An optical study of this SAS-3 error region led to the suggested identification⁹ of a $V = 18.5$ star (4 in Fig. 2) as the counterpart because of its large ultraviolet excess ($U - B = -1.2$ and $B - V = +0.2$).

The existence of correlated temporal behaviour has firmly established the identifications of two of the sources, Cir X-1 (ref. 7) and TrA X-1 (ref. 8). The proposed identification⁹ of 3U1626-67 is probably correct because of the small X-ray error

Table 1 Celestial positions

SAS-3 designation	Other designations	Position (1950)		l^{III} b^{II}	Error radius (90%)	Flux density* (μJy)	Comments
		α	δ				
2S1524-617	TrA X-1 A1524-61	15h24m06.9s 231.°0288	-61°42'41'' -61.°7114	320.3 -4.4	20''	60	X-ray nova ⁴ Optical nova ⁸
2S1516-569	Cir X-1 3U1516-56	15 16 48.6 229.2025	-56 59 14 -56.9872	322.1 0.0	20	25-850	Radio-optical star ⁷
2S1627-673	3U1626-67	16 27 13.6 246.8067	-67 21 23 -67.3564	321.8 -13.1	25	22	7.68-s X-ray pulsar ⁶ Ultraviolet excess star ⁹

*1.0 $\mu\text{Jy} = 2.2 \times 10^{-11} \text{ erg cm}^{-2} \text{ s}^{-1} (2-11 \text{ keV})^{1-2}$.

region and the marked ultraviolet excess of the proposed star. In all cases the identifications were brought about by $\lesssim 1.0'$ X-ray and radio positions^{7,11,17,19} and the optical counterparts have turned out to be quite faint ($B > 17.5$).

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Atmospheric waves in the ionosphere due to total solar eclipse

CHIMONAS¹ predicted that the 'cooling spot' of the lunar shadow of an eclipse, which travels at supersonic speeds through the atmosphere, is a continuous source of atmospheric gravity waves. These gravity waves show up by their interaction with the ionosphere and can be detected using standard radio techniques. Total electron content measurements² in the USA at a distance of several thousand km from the path of totality, detected oscillations which were attributed to a solar eclipse in agreement with the theory of Chimonas³. Measurements made during the 1973 central African eclipse⁴ failed to detect any oscillations, although these results may have been obscured by the occurrence of a magnetic storm at the same time. We present here the results of measurements made on the angle of arrival of a high frequency radio wave inside the path of totality of an eclipse, which took place on 23 October 1976.

The angle of arrival of a continuous wave (CW) obliquely propagated signal on 2.5 MHz was used over a short path of 71.4 km, from Lyndhurst (38°33'S, 145°16'E) to Beveridge (37°28'S, 144°56'E). On 23 October 1976, a total eclipse of the Sun occurred. The radio path was in the path of totality. Two different ionosondes were operated at Beveridge on the eclipse day. A phase path ionosonde, gating at about 100 km, gave group and phase path variations on 2.1, 2.4 and 2.9 MHz and results obtained from this ionosonde indicated that there were always E-region reflections during the eclipse which did not vary by more than ± 5 km. A digitised ionosonde, which records the strongest returned signal, gave ionograms every 45 s. These

ionograms indicated that E_s at a group height of 101 km appeared with a critical frequency $f_o E_s$ of 2.2 MHz at 1619, and at 1621 LT this E_s had an $f_o E_s$ of 2.4 MHz (this is just above the equivalent vertical frequency of 2.35 MHz, assuming a group height of reflection of 100 km). At 1624 LT $f_o E_s$ was 2.2 MHz and at 1625 LT it was 2.7 MHz and gradually increased to over 3.1 MHz at 1630 and remained so until after 1730 LT.

At the start of totality (~ 1642 LT) distinct oscillations with a period of 6-7 min were observed in the angle of arrival. Assuming that the oscillations observed were due to changes in the tilt angle of the sporadic E layer and to a minor extent height variations, it can be shown that the tilt angles along, γ_L , and across, γ_C , the propagation path, are given by

$$\gamma_L = \frac{1}{2} \left\{ \phi' - \frac{\pi}{2} + \tan^{-1} \left(\frac{1}{2 \tan \phi_0 - \tan \phi'} \right) \right\}$$

$$\gamma_C = \tan^{-1} \{ \sin(\theta - \theta_0) \tan \phi \}$$

where $\phi' = \tan^{-1} \{ \tan \phi \cos(\theta - \theta_0) \}$, θ and ϕ are the measured azimuth (bearing) and zenith angles and θ_0 and ϕ_0 are the angles expected for the case of a simple horizontally stratified ionosphere (including a horizontal sporadic E layer). Using the theorem of Breit and Tuve⁵, ϕ_0 was obtained from the group height of the sporadic E layer (assumed to be constant during the relevant period) and γ_L and γ_C were determined. They are shown as a function of time in Fig. 1a and b, several points are

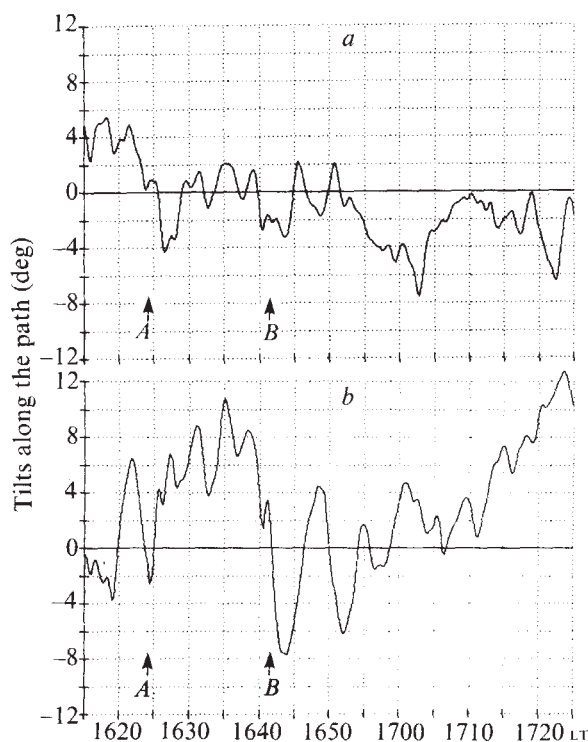


Fig. 1 Variation of the tilt angle of the sporadic E layer with time: a, along the propagation path; b, across the propagation path. A, E_s appeared; B, onset of totality.

evident: (1) between 1625 LT and totality, the E_s layer had an apparent average systematic tilt in γ_L (along the path) of about 6° with a small oscillation superimposed on it. (2) Just after totality this apparent tilt changes considerably and returned to its previous value slowly over about 40 min. (3) During this return, several cycles of a damped oscillation initially of large amplitude occurred of period 6-7 min. (4) No such variations were observed in γ_C (that is across the path).

Between 1619 and 1625 LT, an apparent single oscillation also occurred. As mentioned above, during this period the E_s layer was forming. The digitised ionosonde records indicated that we were only getting strong E_s reflections near 1621–22 LT. The phase ionosonde records indicated that we were still getting E-region reflections near 1619–20 and 1623–24 LT. Since before 1620 reflections from the normal E-region indicated a tilt of $1-2^\circ$, the excursion at 1621–22 is probably due to a reflection from the E_s during its formation.

Chimonas¹ applied his theory to the free bow-wave in the atmosphere outside the penumbra, where the wavefronts of the gravity waves in a previously still atmosphere were expected to be emitted at an angle η to the eclipse where η is given by

$$\tan \eta = V_G/V_E$$

and V_G is the velocity of the atmospheric wave at the altitude concerned (101 km). Taking $V_G = 300 \text{ ms}^{-1}$ and V_E as the velocity of the Moon shadow the wave would be expected to be propagated in a direction $\sim 166^\circ$ south of north (geographic) which is almost along the path between Lyndhurst and Beveridge (it being $\sim 157^\circ$ south of north (geographic)). Hence, outside the shadow, the oscillations due to the eclipse might be expected to be only in γ_L for our geometry. (It might be expected that the changes in the intensity of the solar radiation near totality would cause changes in the electron density gradients and cause changes in the angle of arrival. But, one would expect them to be evident both in the components across and along the path, and there is no reason to expect them to be oscillatory.) The results presented here are interesting since they are in the path of totality, (but south of the centre line), and in fact the oscillations in γ_L decay in amplitude and γ_L returns to near its original value after a few cycles, as may be expected for a fully developed bow wave. But this dominant oscillation only in γ_L inside the shadow is unexpected. The only other angle of arrival measurements, to our knowledge, made during an eclipse⁶ showed that although in the F region a tilt was observed which was consistent with that expected from a consideration of the geometry of the eclipse path, no tilt or oscillation was observed in the normal E region or in an E_s layer which appeared 11 min after the maximum phase of the eclipse and lasted 9 min. These results, however, were not taken in the path of totality, but in a path that experienced only 0.72 obscuration at the maximum phase.

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Charge states of the vacancy in diamond

KNOWLEDGE of radiation damage processes in diamond has increased substantially in the past few years. I present here a concise summary of these data, and suggest that the $25,410 \text{ cm}^{-1}$ (ND1) absorption band occurs at a negatively charged vacancy. It will be shown that with this interpretation, the optical effects of radiation damage fall into a self-consistent pattern.

Optical studies of radiation effects in diamond have been carried out mainly in the visible spectrum following room-temperature irradiation with 1–2-MeV electrons. (For neutron and γ -ray effects see Clark *et al.*¹) Table 1 summarises the results. The models given in the Table are as suggested

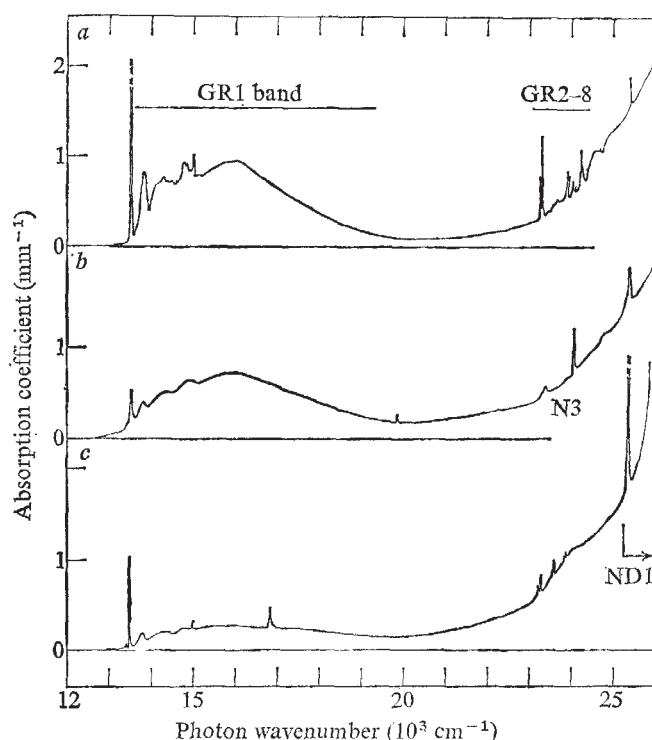


Fig. 1 Visible absorption spectra, measured at liquid nitrogen temperature, of representative diamonds following irradiation at room temperature with 2-MeV electrons to approximately the same dose ($\sim 10^{22}$ electrons m^{-2}). *a*, Relatively pure type IIB diamond, containing about 10^{24} m^{-3} B atoms—the damage level greatly exceeding this³⁶; $G/N = 18$. *b*, Type IA diamond with $4 \times 10^{20} \text{ m}^{-3}$ nitrogen atoms in small aggregates of donor binding energy $E_D \approx 4 \text{ eV}$ ¹⁸; $G/N = 1.3$. *c*, Type IB diamond containing $\sim 5 \times 10^{24} \text{ m}^{-3}$ isolated substitutional atoms of $E_D \approx 1.7 \text{ eV}$ ¹⁶; $G/N \ll 0.1$. For each diamond G/N gives the ratio of integrated strengths (wavenumber $\times$ absorption) of the GR1 and ND1 zero phonon lines. The value for the type IA diamond differs from ref. 37 due to measurement in unfiltered tungsten light. The large linewidths in the type IA diamond are caused by its high nitrogen content³⁸.

previously except that the $25,410 \text{ cm}^{-1}$ (ND1) centre is postulated here to be the negative vacancy, V^- , and not the interstitial nitrogen atom as originally suggested². The reasons for this re-interpretation are as follows. After irradiation, all diamonds show the 'GR' absorption bands (Fig. 1, Table 1), which all occur at the same tetrahedral centre³⁻⁵. Since the GR bands are observable after high temperature annealing ($T > 1,200 \text{ K}$ in relatively pure specimens⁶), it is assumed they occur at the vacancy⁷ because during irradiation, the host interstitial in Group IV semiconductors seems to be highly mobile^{8,9}. The lack of electron paramagnetic resonance (EPR) associated with the GR centre¹⁰ then implies a neutral vacancy V^0 or less likely multiple charged centres (V^{2+} , V^{4+} and so on).

The GR1 band is an E to T transition^{4,5} with its centroid at $(15,920 \pm 150) \text{ cm}^{-1}$ at 30 K. Many-electron LCAO calculations on V^0 predict a 1E or 3T_1 ground state, the two being almost degenerate¹¹⁻¹³. The first allowed transition from 1E , assuming it to be the ground state, is typically at $16,000 \text{ cm}^{-1}$ to a 1T_2 excited state. The excellent agreement with experiment on the irreducible representations of the states, their relative energies and their spin states supports the use of LCAO calculations rather than the alternative techniques which have not yet given even qualitatively correct energy level schemes^{14,15}.

Since the GR bands are the dominant product of irradiating relatively pure diamond (types IIA and IIB, see Fig. 1*a*), it seems that V^0 is the stable form of the vacancy in pure diamond.

It is important to consider the effect of irradiating type IB diamonds, whose dominant impurity is isolated substitutional nitrogen atoms with donor binding energies of about 1.7 eV (ref. 16).

The GR bands are produced only weakly while the 25,410 cm^{-1} (ND1) band, which also occurs at a T_d centre², is extremely strong (Fig. 1c). The simplest explanation is that the vacancies which must be produced by the irradiation are converted from V^0 to V^- by charge transfer from the nitrogen donors: Loubser (personal communication) has observed a decrease in the EPR of these donors as the irradiation proceeds.

LCAO calculations, which seemed to be accurate for V^0 , predict that the first absorption band of V^- should occur between about 20,000 and 38,000 cm^{-1} in an 4A_2 to 4T_1 transition¹¹⁻¹³. Experimentally the ND1 transition has been found to occur from an A to a T state², the centroid of the band being at $(27,500 \pm 200) \text{cm}^{-1}$. The theoretical 4A_2 ground state implies an EPR active centre. No quantitative experimental study of this point has yet been made. A single isotropic line with $g \approx 2.0$ has been observed in the three specimens inspected here, and also in measurements by Dyer and du Preez¹⁷. This EPR and the ND1 optical absorption show strikingly similar cyclic behaviour when the diamonds are subjected to alternate heating and bleaching cycles. However, Dyer and du Preez observed a decrease in the EPR signal and an increase in ND1 absorption after a 500 °C anneal in one specimen, suggesting that the absorptions arise at different centres, but these com-

parisons are not straightforward in view of the photochromic behaviour of the ND1 centre¹⁸. A detailed study of this EPR is required.

The assignment of the 25,410 cm^{-1} (ND1) band to V^- produces two simplifications. First, reversible photochromic effects occur between the 13,495 cm^{-1} (GR1) and 25,410 cm^{-1} (ND1) bands even when the vacancy concentrations are $\lesssim 10^{-3}$ of the impurity content of the diamonds^{17,18}. If GR1 and ND1 occur at chemically indistinguishable sites a mechanism of charge transfer between defects of minor concentrations has to be designed. In terms of the present model, photoionisation of V^- (ND1) leads necessarily to V^0 and hence GR1 absorption. Presumably the released electron produces the observed photoconduction¹⁹. Conversion of GR1 (V^0) to ND1 (V^-) by blue light¹⁸ ($23,250 \text{cm}^{-1} < h\nu < 25,400 \text{cm}^{-1}$) is then seen to be by hole emission ($V^0 \rightarrow V^- + h^+$), explaining the photoconductivity observed experimentally²⁰.

Second, in nitrogen-containing diamonds, thermal annealing destroys the 13,495 and 25,410 cm^{-1} bands (GR1 and ND1) producing new optical bands (for example, at 15,690 and 19,865 cm^{-1}) depending on the form of nitrogen^{21,22}. Recent data imply that the new bands are formed when the nitrogen traps a vacancy²³⁻²⁵. In some experiments (ref. 23 and M. H. Nazaré, personal communication) the rate of growth of the new bands matches the destruction of GR1, in others^{22,26} it matches the removal of ND1. This is rationalised if both GR1 and ND1 occur at centres of the same chemical species (such as V^0 and V^-), and if we assume a charge redistribution can occur, if necessary, after trapping.

All our present optical data on irradiated diamond seem to be consistent with ND1 being V^- . In principle, this model can be tested using a photo-Hall experiment to identify the carrier emitted when GR1 and ND1 centres are photoionised. The model predicts

$$h\nu + \text{GR1} (V^0) \rightarrow \text{ND1} (V^-) + h^+ \quad (1)$$

$$\text{and} \quad h\nu + \text{ND1} (V^-) \rightarrow \text{GR1} (V^0) + e^-$$

In contrast, if GR1 and ND1 occur at chemically different sites, the same charge must be liberated when the GR1 and ND1 centres are photoionised, so that the observed cyclic processes can occur. The available data are not inconsistent with equation (1)²⁷.

The earlier suggestion² that the ND1 centre was an interstitial nitrogen atom was reached by arguing that defects in diamond have highly localised orbitals. In this limit it was thought reasonable to 'assume that it is unlikely that the effect of the presence of nitrogen (as an impurity in a diamond) is to produce charge compensation'². However, the data now available for type IB diamond (see Fig. 1c) show that the absorption strength of chemically stable defects like the GR1 centre is in fact strongly affected by the presence of the deep nitrogen donor ($E_D \approx 1.7 \text{eV}$), an effect most likely to occur as a result of charge compensation.

Finally, the fate of the carbon interstitials, produced by the irradiation, seems to be that they are highly mobile, as in silicon²⁸, and become trapped at unknown crystal defects, producing complex EPR centres²⁹.

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Table 1 Major visible absorption bands

Optical band* (cm^{-1})	(eV)	Nature of transition†	Ref.‡
10,080 H2	1.25	End product of annealing radiation damage in diamonds containing nitrogen	21
13,495 GR1	1.673	$E \rightarrow T$ at T_d centre V^0	4, 5
13,590 15,000 15,690 16,825	1.685 1.860 1.945 2.086	A $\rightarrow$ A transitions at a tetragonal centre? A $\rightarrow$ E at trigonal centre V-N End product of annealing radiation damage in diamonds containing nitrogen; complex response to uni-axial stress	30 23 §
17,390 19,495	2.156 2.417	$E \rightarrow A$ at trigonal centre <110> electric dipole at monoclinic I centre	31 32
19,860 3H	2.462	<110> electric dipole at C_{2v} centre	33
19,865 H3	2.463	<110> electric dipole at C_{2v} centre N-V-N	34 25
20,150 H4	2.498	<110> electric dipole at C_{1h} centre; V plus an aggregate of nitrogen	32
20,510 21,280 TR12	2.543 2.638	V-V? σ dipole at monoclinic I centre	21 33
23,240 23,295 GR2, GR3	2.881 2.888	$E \rightarrow T$ transitions at 13,495 cm^{-1} centre (V^0)	3
23,410 23,710 23,860 24,000 24,200 GR4,5,6,7,8	2.902 2.940 2.958 2.976 3.00	$E \rightarrow ?$ transitions at 13,495 cm^{-1} centre (V^0)	3
23,550 24,080 N3	2.920 2.985	$E \rightarrow A$ transition at trigonal centre A $\rightarrow$ E transition at C_{3v} centre, probably 3 nitrogen atoms	31 35
25,410 ND1	3.150	A $\rightarrow$ T transition at T centre (V^-)	2 See text

*Bands are labelled by their zero phonon lines, in cm^{-1} and eV, and by their common names.

†The order of the irreducible representations for the transitions is ground $\rightarrow$ excited state.

‡Only the latest relevant references are given.

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Alkali cations and direct reduction of wustite

THERE are few reports^{1,2} on the direct reduction of wustite by carbon according to an autocatalytic mechanism, and none deal with its quantitative analysis in terms of any kinetic model. Eck³ has postulated that this mode of reduction is indicative of the increased role of processes such as diffusion and nucleation in the solid state, relative to the gasification of carbon by the Boudouard reaction—generally regarded as the rate-controlling step in the reduction⁴. We describe here a quantitative analysis of autocatalytic reduction in terms of the Prout-Tompkins⁵ mechanism for the growth of a product phase in the lattice of the unreacted phase. We derive a rate constant characterising the growth of the metallic phase and use it to evaluate the catalytic effects of small admixtures of alkali salts (ref. 6 summarises the literature on catalysed reduction).

Reduction of wustite in mixtures comprising precipitated red ferric oxide (Hopkin and Williams), spectrographic graphite (Union Carbide) and varying amounts of alkali salts (cation/Fe from 0.5% to 3.6% depending on catalytic effect) was studied at constant temperature by a Mettler Thermoanalyser. Samples were heated from room temperature at a rate of 25 °C min⁻¹ to the pre-selected temperature; simultaneous values of temperature, cumulative mass change (TG) and rate of mass change (DTG) were recorded.

The start of the wustite reduction (which always occurred after the reaction temperature was reached) was fixed at that value of mass loss which corresponds to the complete conversion of ferric oxide to wustite, assuming it to take place in a self-generated CO/CO₂ atmosphere in equilibrium with Fe₂O₃/Fe₃O₄. X-ray diffraction analyses of partially reacted samples confirmed the two-stage nature of reduction: Fe(III)—and Fe(III, II)—oxides were no longer detected when metallic iron appeared in wustite. The reductions were all carried to completion (as confirmed by stoichiometry and cessation of mass loss). Fractional conversion (α) of wustite to metallic iron was calculated from the TG data as fractional mass change values. Representative reduction curves are shown in Fig. 1.

The average mass change (29 measurements) was 38% relative to the starting mass of wustite. This suggests that >90% of wustite oxygen was abstracted as CO and that the composition of the reduction atmosphere lay closer to the Boudouard equi-

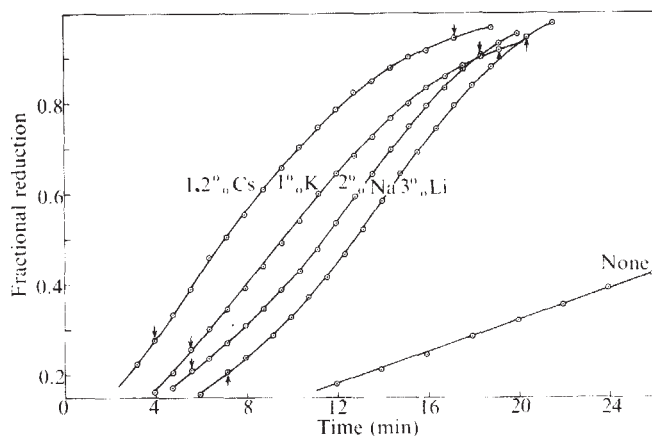


Fig. 1 Reduction of wustite to metallic iron by graphite in a dynamic argon atmosphere (flow rate 50 ml STP min⁻¹) at 960 °C in the presence of alkali catalysts. Alkalis were added as dry powders in the form of carbonates except for Rb (nitrate). TG data points were hand-read from a chart recording for the construction of reduction curves. Samples mass: 80 mg Fe₂O₃+15 mg C. Particle sizes: FeO ~ 1 µm (by scanning electron microscopy); graphite, 144+105 µm. Sample container: cylindrical alumina crucible (length, 20 mm; diameter 8 mm) resting on a Pt/Rh thermocouple junction. Sample loose-packed (bulk density ~ 0.5 g cm⁻³). Arrows indicate range of α for which the curves conform to equation (1).

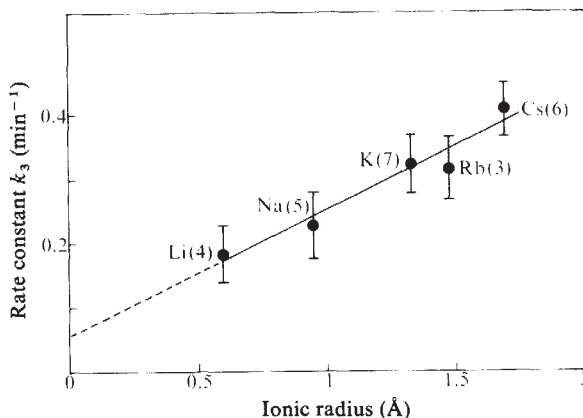
librium (~ 98% CO) than to the wustite-iron-gas equilibrium (~ 70% CO).

We have estimated the rate constants characterising the reduction curves of Fig. 1 by fitting the curves in the range $0.2 < \alpha < 0.95$ to a modified form of the Prout-Tompkins equation for nucleation phenomena. The modification takes into account our experimental observation that the curves are in general not symmetric

$$\ln \frac{\alpha}{1 - (\alpha/2\alpha_1)} = k_3 t + \text{constant} \quad (1)$$

α_1 is the value of α for which the rate of reduction (traced by the DTG curve) attains its maximum. k_3 is the so-called branching coefficient describing the appearance of nucleated iron by a

Fig. 2 Empirical relationship between the Prout-Tompkins branching coefficient k_3 and the Pauling ionic crystal radii of alkalis. Numbers in parentheses indicate the number of determinations of k_3 . Error bars denote the 95% confidence intervals (Student's *t* distribution). Line through data points is the least-squares fit. a (intercept) = 0.055 ± 0.025 , m (slope) = 0.195 ± 0.021 . Errors correspond to 1σ . The best-fit value of intercept is identified with the uncatalysed value of k_3 : $0.066 \pm 0.016 \text{ min}^{-1}$ (four determinations).



chain mechanism. (See ref. 5 for the derivation of equation (1) when $\alpha_1 = 0.5$.) In this interpretation of reduction, autocatalysis is essentially a consequence of the proportionality of the rate of reduction to the number of iron nuclei. Justification for this proportionality is provided by observations⁷ that the rate of abstraction of oxygen from wustite is much higher at the triple phase boundary wustite-iron-gas than elsewhere. We interpret deviations from the Prout-Tompkins mechanism for $\alpha < 0.2$ as resulting from the significant contribution made by the (unknown) initial nucleation law to the formation of metallic iron; derivation of equation (1) assumes this contribution to become negligible at some (low) value of α .

A summary of k_3 values derived by means of equation (1) (and recalculated by linear interpolation or extrapolation at cation/Fe = 2% to facilitate comparison) is given in Fig. 2. This also demonstrates a linear relationship ($r = 0.972$) between the catalytic effects of alkali cations and their ionic sizes.

We conclude first, that autocatalytic reduction of wustite yielding sigmoid-shaped reduction curves can be explained by invoking the Prout-Tompkins (branching chains) mechanism for the formation of metallic iron; and second, that alkali cations enhance the rate of this mechanism in direct proportion to their ionic sizes.

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Pacific sea-surface temperature related to rain in California

CALIFORNIA is experiencing the worst drought in modern history, and there is much concern and speculation about future rainfall prospects. We recently succeeded in relating South Atlantic sea surface temperatures (SST) to rain in northeastern Brazil¹, and here we use similar methods to relate sea surface temperatures over the North Pacific Ocean to rainfall in California. We found December SST in the north-east Pacific off Washington to have a limited but definite predictive value, and to account for about 45% of the variation in California rainfall for the period February-April.

Namias has published several papers relating SST in the Pacific to atmospheric circulation². In our approach, aimed at obtaining a useful forecasting tool, we go directly from a contributory cause (SST) to an ultimate effect (rainfall), bypassing intervening physical processes. We justify this by the belief that rainfall is an integrator of the multitude of ocean-atmospheric interactions and processes that occur simultaneously on several scales of space and time.

We divided the rainy season into two parts, early rains (November-January), and the late rains (February-April). The months May-October are usually dry in California,

and when rains do occur they are influenced by factors apart from the normal November-April rains. We did not investigate the effect of SST on rains in May-October, although SST off Baja California is almost certainly related to the occurrence of September and October rains originating from decaying *chubascos*, or west coast Mexican hurricanes.

First, we established rainfall indices (RFI) for California. Rainfall is quite highly correlated among stations over most of the state. We chose three stations: Sacramento, Fresno and Los Angeles. All have long records, and are about equally spaced along the length of California. All three stations respond only to synoptic situations that are definitely wet, and receive little moisture from marginal situations. For November-April all three stations have high correlation with the November-April RFI, r being 0.90, 0.92 and 0.89, for Sacramento, Fresno and Los Angeles, respectively. To give each station equal weight in the index, multipliers of 0.86, 1.26 and 0.96, derived from 1930-60 mean annual rainfall values, were used for Sacramento, Fresno and Los Angeles, respectively. The RFI for a given period is the sum of weighted rainfall for that period, in millimetres.

Interestingly, for the period 1889-1972 there is no correlation between early and late rainfall indices. Kendall's parameter τ equal to -0.07 and r to -0.02, are not significant. Thus failure of early rains does not indicate that late rains will also be deficient.

Our SST values were mean temperatures developed by NMFS Pacific Environmental Group from ship observations assembled by the National Climatic Center. These values are means of all observations taken in a given 5° quadrant in a Marsden Square, during a given month and year. They have not been corrected for space or time bias, and it would be expected that correlations obtained from them would be improved if corrections could be made. We used monthly averages of available ship observations of SST in 5° quadrants, 1931-72, for the Pacific Ocean north of 15°N latitude. Ship reports for three Marsden Squares (90, 128, 130) were not conveniently available, so these areas were not considered. The area to the south-east of Hawaii and equatorwards of 15° is probably influential in California rainfall, but SST data there are too sparse for analysis. Elsewhere, data exist in most quadrants for most years, except 1941-46. In preparing our correlation maps, we used all values that were averages of four or more observations in a given month and quadrant, except a few extreme coldest and warmest values based on less than 10 observations were excluded.

In our work relating rain in Brazil to SST in the South Atlantic Ocean we found a slight warming trend of 0.0078 °C per yr for the period 1907-72, and had assumed that this warming was due to instrumental error resulting from evaporative cooling from bucket samples in early years, and heating by ships' engines in injection temperatures in later years. Removal of this trend improved correlations with rainfall. We had originally intended to adjust the North Pacific SST data for the same reason, but soon found that the trend was generally much larger and more variable in space and time than could be accounted for by instrumental factors. We therefore added an arbitrary 0.01 °C per yr before 1972, to compensate partially for the warming trend. Correlations obtained when temperatures are so adjusted are higher than when the raw data are used, which suggests that some degree of instrumental warming may be real.

We found little significant or useful correlation between Pacific SST and November-April rainfall, although there is a tendency towards negative correlation extending from the central Pacific to the region south-east of Japan. In general, the cold water there tends to make California rainy.

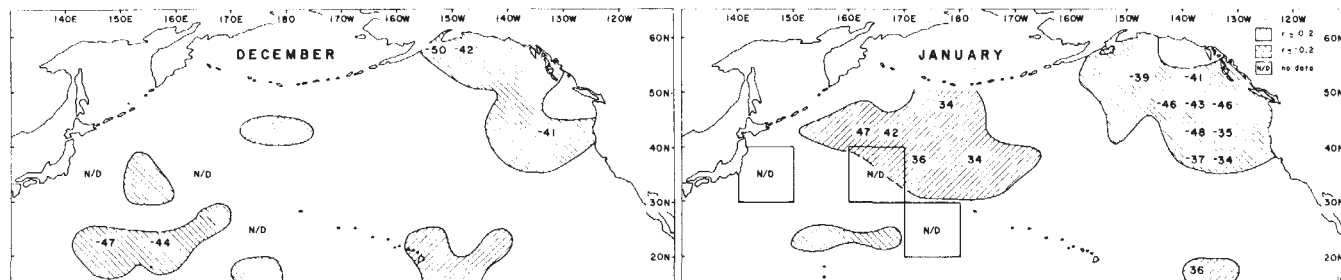


Fig. 1 Correlation between SST and November-January rainfall in California. Correlation coefficients plotted when significant above the 0.05 level.

We found no useful forecasting relation between Pacific SST and November-January rainfall, however, these rains are accompanied by negative correlation—cold water off the coast (Fig. 1). This substantiates Namias' view that warm water off the coast was involved in the 1976-77 winter drought in California³.

A useful forecasting relation seems to exist between SST and February-April rainfall. The situation begins with negative correlation (cold water) in the western and central parts of the Pacific in October. Later in November, December and January, positive correlation (warm water) appears in the Gulf of Alaska. This progresses southwards in February and March, while cold water remains a short distance out to sea (Fig. 2). The area of positive correlation in the north-east Pacific off Washington in December seems to offer the most promise in forecasting February-April rains. In this area MS 158-4 has a correlation coefficient (r) of 0.63 between December SST and February-April rainfall, significant above the 0.001 level. It seems that processes induced by cold water in the central and western Pacific in October, November and December,

when followed by warm water west of Washington in December, are favourable to February-April rains in California. This implies that SST gradient between the two areas is important although we found that this gradient does not yield correlation coefficients as high as using SST at MS 158-4 directly.

Mean SST values for a given month and quadrant contain real SST variations, as well as considerable noise due to non-random distribution of observations in space and time and to various instrumental errors. This noise can be reduced by using a median anomaly from three adjacent quadrants, using those quadrants most nearly aligned with the isotherms of SST. The median is a better measure than the mean because of the effect of extreme values on the mean. The three-quadrant median deviation is taken as the corrected deviation from normal for the central quadrant. Table 1 illustrates the derivation of corrected SST values for MS 158-4.

When monthly SST values in MS 158-4 are refined as stated above, by introducing data from adjacent quadrants, $r=0.68$ and $\tau=0.50$, both with massive significance. This

Fig. 2 Correlation between SST and February-April rainfall in California. Correlation coefficients plotted when significant above the 0.05 level.

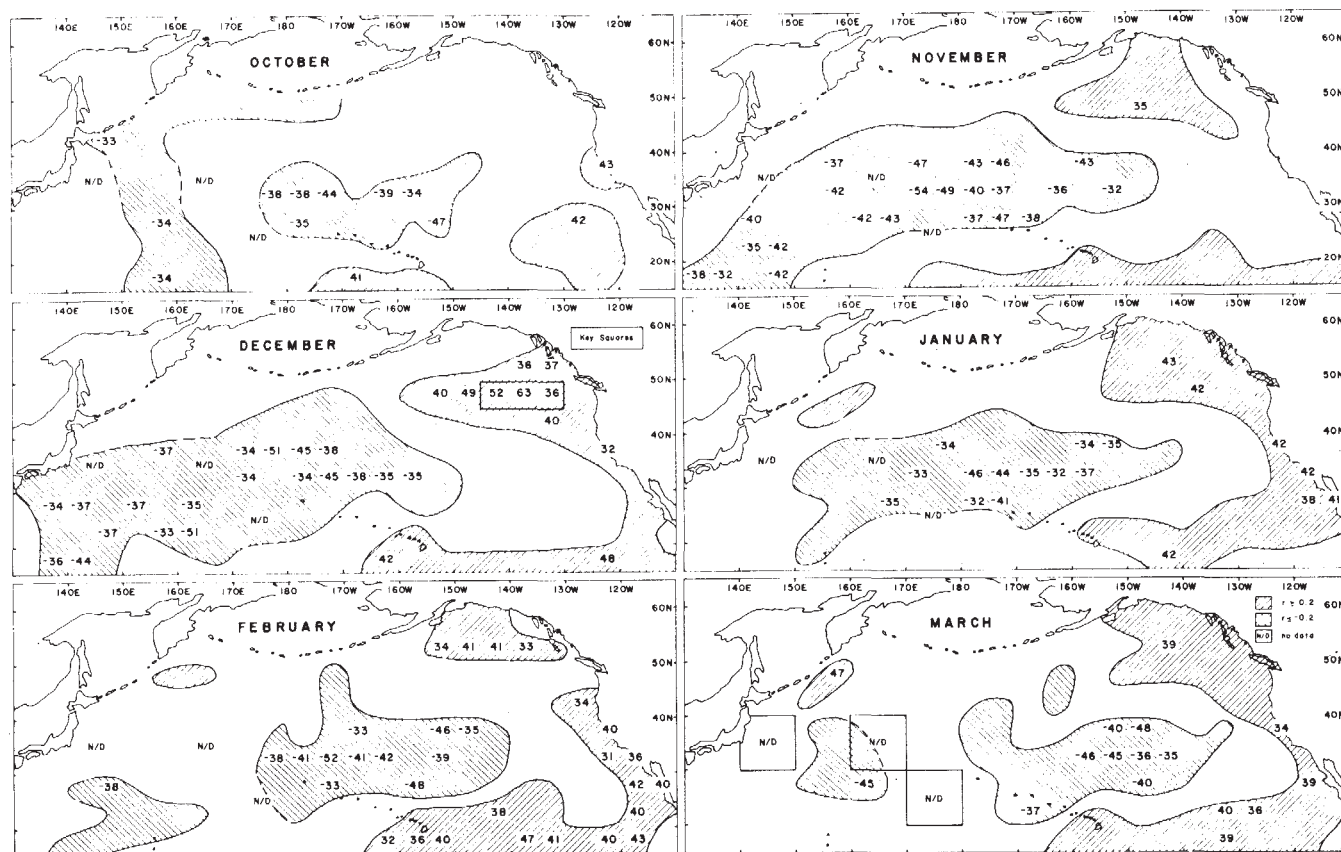


Table 1 Derivation of corrected SST for MS 158-4 ($^{\circ}\text{C} \times 100$)

Year	SST for MS 158-4		Deviation	Deviation	Deviation	Correction†	Corrected SST MS 158-4
	Raw value	Adjusted*	MS 159-3	MS 158-4	MS 158-3		
1931	824	865		-48	22		
32	750	790	-126	-123	-7	0	790
33	736	775	-80	-138	-110	28	803
34	900	938	94	25	62	37	975
35	894	931	14	18	95	0	931
1936	1018	1054	158	141	154	13	1067
37	1029	1064	212	151	-42	0	1064
38	797	831	116	-82	-154	0	831
39	829	862	58	-51	-76	0	862
40	900	932	35	19	43	16	948
1941	—	—	—	—	—	—	—
42	—	—	—	—	—	—	—
43	—	—	—	—	150	—	—
44	—	—	—	—	190	—	—
45	916	943	116	30	-28	0	943
1946	—	—	165	—	—	—	—
47	—	—	—	—	—	—	—
48	—	—	-93	—	-209	—	—
49	947	970	8	57	94	0	970
50	897	919	-65	6	-91	-71	848
1951	906	927	-24	14	-38	-38	889
52	843	863	-44	-52	7	6	869
53	831	850	-39	-63	-27	24	874
54	877	895	-46	-18	-32	-14	881
55	823	840	2	-73	-90	0	840
1956	844	860	29	-53	-32	21	881
57	1034	1049	209	136	137	1	1050
58	953	967	-3	54	51	-3	964
59	1012	1025	33	112	75	-37	988
60	848	860	-120	-53	104	0	860
1961	1078	1089	12	176	70	-106	983
62	1079	1089	-22	176	35	-141	948
63	787	796	-35	-117	-21	82	878
64	958	966	-93	53	-41	-94	872
65	878	885	-44	-28	10	0	885
1966	901	907	8	-6	-11	0	907
67	798	803	-125	-110	-78	0	803
68	871	875	-105	-38	-22	0	875
69	867	870	-63	-43	-8	0	870
70	878	880	-70	-33	-67	-34	846
1971	845	846	-52	-67	-93	0	846
72	948	948	-21	35	-22	-56	892
<i>n</i>	35	35	36	35	38		34
Mean SST	894	913	862	913	996		904
<i>r</i>	0.593	0.628					0.668
<i>t</i>	4.23	4.63					5.07
α''	<0.001	<0.001					<0.001
ϕ	0.441	0.482					0.509
<i>z</i>	3.73	4.07					4.24
α''	<0.001	<0.001					<0.001

Median values are italicised.

*0.21 $^{\circ}\text{C}$ added for each year before 1972.

†Difference between MS 158-4 deviation from normal and three square median deviation from normal.

provides the basis for a February–April rainfall forecast using December SST (Fig. 3). Normal is defined as the mean for the 34 yr during the period 1931–72 when data exist in all three quadrants. When SST is normal, the rainfall forecast will be for 100% of normal, with 95% confidence limits between 10% and 190% of normal. If SST is 1 $^{\circ}\text{C}$ above normal, the rainfall forecast is for 160% of normal, with 95% confidence limits between 65% and 250%. If SST is 1 $^{\circ}\text{C}$ below normal the rainfall forecast is for 40% of normal, with 95% confidence limits between 0% and 135% of normal. The confidence limits are unfortunately wide, but probably reflect with reasonable accuracy the limits set by SST upon rainfall. Although imprecise, it is an improvement over a forecast based on climatic normals, which would be for 100% of normal with 95% confidence limits between 10% and 190% of normal, regardless of SST.

The τ value of 0.50 implies that the odds are three to

one that a change in rainfall will match the sign change in SST.

We believe that, early in the season, cold water in the central Pacific induces greater-than-normal troughing in the overlying atmosphere. This induces an increased frequency of south-westerly winds that push the SST isotherms northwards in the Gulf of Alaska. As winter advances the cold anomaly in the central Pacific is advected slowly eastward. Troughing in the atmosphere may likewise move eastward, and act to decrease the intensity of the surface subtropical high pressure cell off the California coast. The resultant decreased north-westerly winds along the coast allows a warm SST anomaly to form by decreased upwelling and by reduced southward flow of the California Current. The warm water moves southward, and then westward with the North Equatorial Current as the season advances, to create a positive anomaly of SST near Hawaii. Namias has shown that warm water in this area is important for

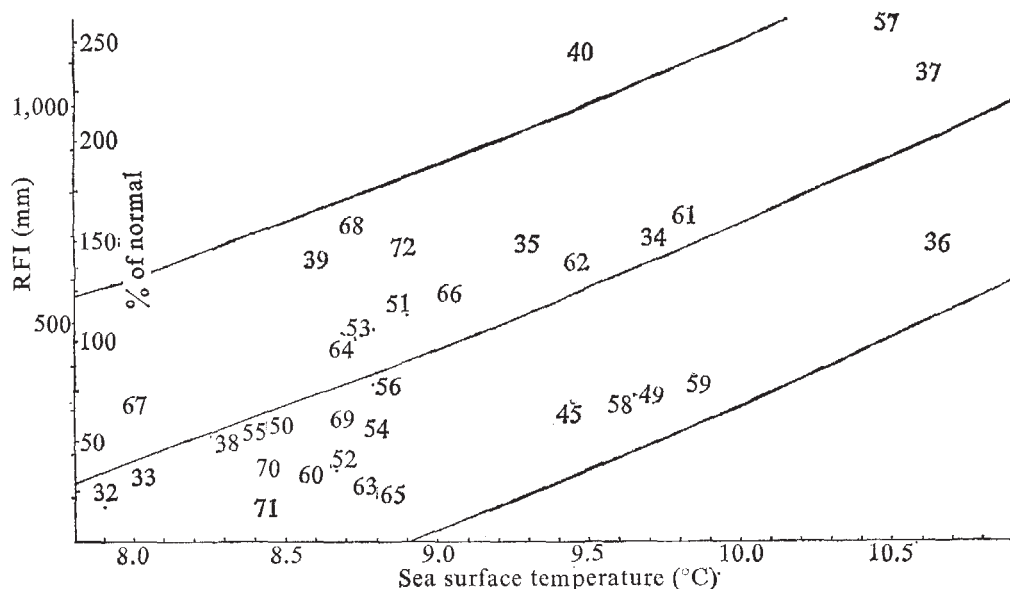


Fig. 3 February–April California rainfall indices (RFI) plotted against corrected December SST in Marsden Square 158–4. Normal SST is 9.04 °C and RFI is 459 mm. Years indicated are for SST. Second degree regression line with 95% confidence limits is shown.

California moisture⁴. Our speculation is tenuous, but we hope that other researchers will study the cause and effect relationships underlying what seems to be real correlation between SST and California rainfall.

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Direct measurements of secondary currents in river bends

FLUIDS flowing through pipes or channels can develop secondary currents. These are defined as currents which occur in the plane normal to the local axis of the primary flow. Their development in straight channels has been ascribed to anisotropic turbulence and the non-uniform distribution of boundary shear stress^{1–3} but in meander bends they are generally caused by skewing of the flow^{3–5}. Secondary currents distort the distributions of primary isovels and boundary shear stress from those expected in simple flows and, therefore, have important implications for bed and bank erosion and for resistance to flow. We report here measurements of longstream and cross stream velocities carried out across sections of a river perpendicular to the outer banks of several bends using an electromagnetic flow meter.

At meander bends fully developed secondary currents appear as a single flow cell, carrying surface water towards the outer

bank and bed water towards the inner bank^{6–9}. Measurements in flumes^{10,11}, however, and in rivers^{12,13} have indicated the existence of a small cell of reverse circulation at the outer bank. A similar feature seems to exist in pipes¹⁴. As this reverse cell has been observed at single bends it is not connected with the reverse circulation of a relict cell from an upstream bend^{15,16}. Rozovskii¹¹ concluded that it is a wall effect, extending over a region of perhaps one or two depths from the bank, but negligible in bends with large ratios of width to depth. Hey and Thorne¹² proposed a new theory of secondary flow at bends, incorporating two cells in place of the classical single cell.

Identification of the outer bank cell in river bends has been based on only a few direct measurements of secondary flows^{12,13}. Because of a lack of suitable instrumentation, these measurements were relatively inaccurate and more detailed information is therefore required. The development of the electromagnetic flow meter has enabled such measurements to be made. This meter measures velocity in two directions simultaneously and so enables the components of horizontal flow to be defined. Measurements can be made to within 1 cm s⁻¹ (refs 17 and 18).

In this investigation, measurements were made with an electromagnetic flow meter along sections perpendicular to the outer banks of several bends. The meter was positioned to measure the longstream velocity (parallel to the outer bank) and the cross stream velocity (perpendicular to the outer bank.) These are not necessarily the primary and secondary velocities but the following theory allows calculation of those components.

Secondary currents at any vertical through the flow are defined to occur in the plane normal to that containing the mean primary velocity vector at that vertical. It is assumed that in steady conditions, the average secondary flow in the plane is zero, that is, the secondary discharge in one direction is balanced by the discharge in the other. The longstream and cross stream velocities (those to which the electromagnetic meter responds) are defined by u and w respectively. The resultant velocity q at any depth y is defined to be at a horizontal angle θ to the longstream axis so that

$$u = q \cos \theta \quad (1)$$

$$w = q \sin \theta \quad (2)$$

The mean primary velocity U at the vertical is at the fixed angle I to the longstream axis. I defines the vector of the

primary flow so the primary velocity U_p at any depth is found by resolving in that direction

$$U_p = q \cos(\theta - \phi) = u \cos \phi + w \sin \phi \quad (3)$$

The secondary velocity U_s is obtained by resolving in the plane normal to that containing the primary velocity vector

$$U_s = q \sin(\theta - \phi) = w \cos \phi - u \sin \phi \quad (4)$$

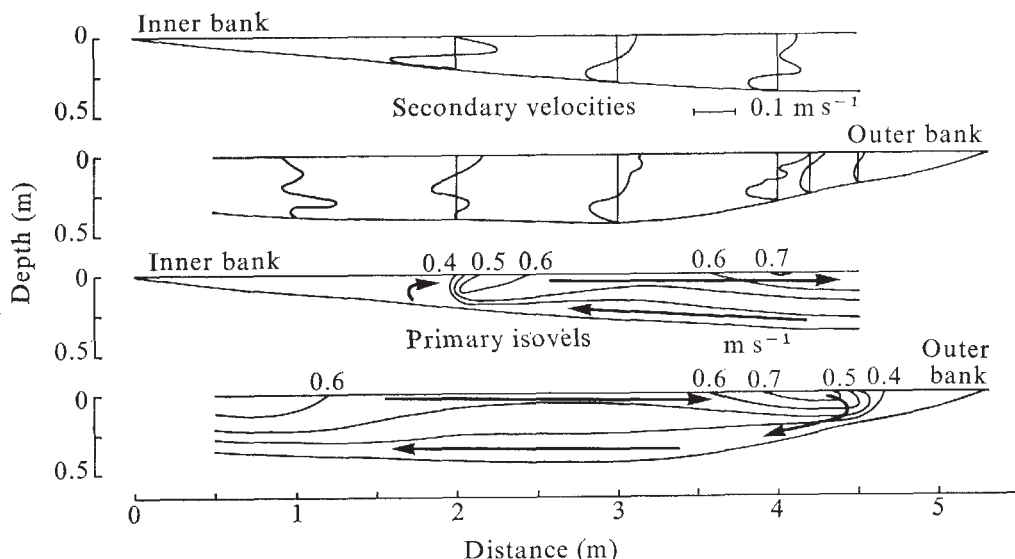
ϕ is unknown but is found by integrating the secondary

as far as was necessary to establish the presence of the main cell (Figs. 2 and 3).

The single classical cell does not always continue to the outer bank. Reverse cells are evident up to one depth from the bank at Rickety Bridge Bend and at Maes Mawr Bend. This pattern is reflected in the distortion of the primary isovels. However, the outer bank cells do not appear at Llandinam Bend.

The outer bank cells probably result from interaction of the main cell with the outer bank. The flow of this cell has a component perpendicular to the bank and, in an ideal fluid, the boundary streamlines of this component would follow exactly the water surface and the boundary of the channel. In a real fluid, this is not possible since it requires an infinite acceleration

Fig. 1 Primary and secondary velocities at Llandinam Bend—River Severn. Channel width 10 m; radius of curvature 75 m; bend arc angle 62° ; discharge $1.1 \text{ m}^3 \text{ s}^{-1}$.



velocities over the depth, d . As their mean is defined to be zero,

$$\int_0^d q \sin(\theta - \phi) dy = 0 \quad (5)$$

so that

$$\tan \phi = \frac{\int_0^d q \sin \theta dy}{\int_0^d q \cos \theta dy} = \frac{\int_0^d w dy}{\int_0^d u dy} \quad (6)$$

w and u are the velocities measured by the electromagnetic meter. Substitution of the value of ϕ into equations (3) and (4) allows the secondary and primary velocities at any depth to be calculated.

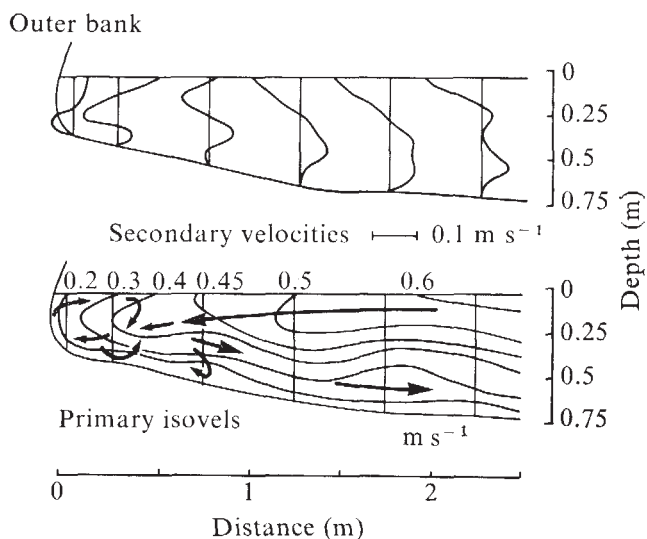
Measurements were made at three bends on the Upper River Severn. Llandinam Bend is a single bend at a shallow section of channel. Rickety Bridge Bend is a slight bend just downstream from a riffle which directs the flow strongly against the outer bank. Maes Mawr Bend is a conventional meander bend, on a pool and downstream of a shallow reach.

A complete survey was made at Llandinam Bend at a low discharge and this shows the classical cell across the whole channel (Fig. 1). At the other sites, velocity profiles were taken at progressively greater distances from the outer bank but only

at the junction of bank and water surface, so a breakdown of the ideal pattern is expected.

The occurrence of the reverse cells seems to depend on the form of the bank. They do not occur where the bank shelves but do where the bank is perpendicular to the water surface. This is verified by several other surveys made at high and low discharges and at shelving and steep banks at Maes Mawr bend. Where the outer bank is steep, the junction

Fig. 2 Primary and secondary velocities at Rickety Bridge Channel—River Severn. Channel width 8 m; radius of curvature 50 m; bend arc angle 50° ; discharge $1.7 \text{ m}^3 \text{ s}^{-1}$.



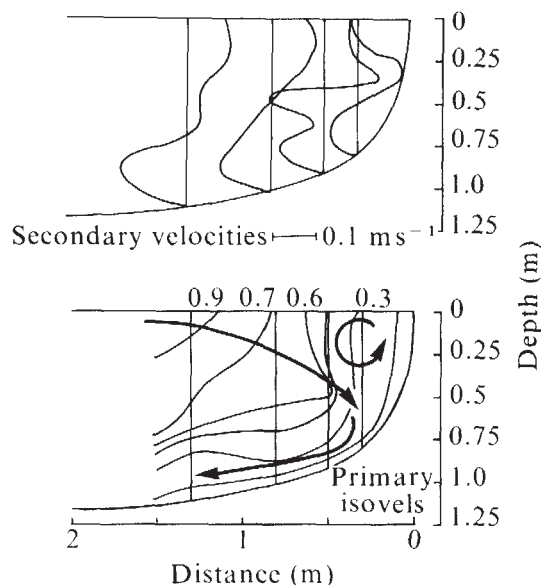


Fig. 3 Primary and secondary velocities at Maes Mawr Bend—River Severn. Channel width, 21 m; radius of curvature, 70 m; bend arc angle, 38° ; discharge $9 \text{ m}^3 \text{ s}^{-1}$.

of bank and water surface (seen in section) corresponds to a stagnation point. At this point, water would be expected to pile up and it is possible that the resulting upwelling at the surface, combined with the weight of the superelevated water, could trigger a more general reversal of secondary flow in the region of the bank. This process might be aided by the local surging of flow which results from the passage of turbulent eddies.

If this theory is correct, the strength of the outer bank cell should be proportional to the component of flow towards the outer bank. This seems to be confirmed because the cell at Rickety Bridge Bend, where the flow is directed against the bank, is stronger than at Maes Mawr Bend.

Direct measurements of outer bank cells confirm that the classical cell oversimplifies the pattern of secondary flow in some river bends. This has important repercussions for the evaluation of flow resistance, the distribution of boundary shear stress, the movement of bed load and thence bank erosion and meander migration.

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Prehistoric dental calculus gives evidence for coca in early coastal Ecuador

LOS CERRITOS, a late Chorrera (Engoroy) cemetery on the Santa Elena Peninsula of Ecuador yielded artefacts and radiocarbon assay which indicate that use of the site began about 840 BC and continued for several years within the Late Formative¹. The human skulls from the site are of particular interest because many showed heavy accumulations of supragingival dental calculus (tartar), which contrast with the relatively slight calculus accumulations on the teeth of skulls recovered from the earlier Valdivia (3000–1500 BC) site of Real Alto², also on the Santa Elena Peninsula. We argue here that the heavy calculus accumulations are the result of habitual, secular coca chewing in late Chorrera times. This diagnosis, in turn, supports the hypothesis of prehistoric trade in coca, probably in exchange for mollusc shells, with inhabitants of the eastern Andean slopes.

Of 27 adult human skulls from Los Cerritos with dentitions sufficiently intact to be evaluated, 17 had calculus deposits which ranged from slight to extreme (Table 1). This scale was devised to accommodate the range of variation in the material; even 'slight' would be considered heavy by modern standards.

According to ethnographic accounts^{3–5}, coca leaves were commonly chewed with lime in western South America. The lime is responsible for the dental deposits. Mehta *et al.*⁶ noted that heavy calculus in modern India correlated with chewing betel with lime. Leigh⁷ reported large dental accretions from prehistoric Guam, which he attributed to the habit of chewing betel nut with lime. He concluded that similar deposits from prehistoric Peru resulted from coca chewing⁸. Moodie⁹ also observed excessive calculus among pre-Columbian Peruvians. In the prehistoric examples, as in the Chorrera material, the deposits were on the buccal tooth surfaces of both maxilla and mandible, which is in accord with the practice of holding the quid between the cheek and teeth. In contrast, the sites most affected in modern populations are those near the openings of the salivary ducts—the lingual surfaces of mandibular incisors and the buccal surfaces of maxillary molars.

Lime has been implicated as an aetiological factor in dental deposits, but the nature of the deposits is uncertain. Mehta *et al.*⁶ called the deposits calculus, but Leigh⁸ referred to them as lime. The chemical composition not only provides an accurate description of the pathology, but can reveal clues about the mechanism and conditions of the depositions. Six samples of dental accretion from six Los Cerritos skulls were subjected to elemental and X-ray diffraction analysis. The samples were cleaned only by dry-brushing.

The major and minor elemental analysis of the deposits was performed by standard X-ray fluorescence techniques as developed by Rose *et al.*¹⁰. To approach a total composition of the material mean values are reported in Table 2 as the percent of oxides, even though the elements actually occur in mineral form. The sum of the components is less than

Table 1 Calculus deposits on the teeth of adult human skulls from Los Cerritos

Extent of accumulation	Probably male	Probably female	Sex indeterminate
Extreme	1	0	0
Very heavy	4	0	0
Moderately heavy	5	2	0
Slight	1	4	0
None	2	7	1

100% because very limited sample sizes precluded determination of carbon dioxide, moisture, and ignition loss.

X-ray diffraction patterns of each sample revealed a poorly-crystalline apatite-like phase representing hydroxyapatite, $\text{Ca}_{10}(\text{OH})_2(\text{PO}_4)_6$, a major component of calculus¹¹⁻¹³. Considerable quartz was also present, together with some calcium silicate, $\text{Ca}_3\text{Si}_2\text{O}_7$, or calcium aluminium silicate, $\text{CaAl}_2\text{Si}_2\text{O}_8$. The similarity of the compositions indicates that the teeth were in contact for long periods with ground water from which silica precipitated within the relatively porous calculus structure.

Table 2 Major and minor elemental analysis of dental deposits

Element	% of oxides
SiO_2	40.02 ± 10.78
Al_2O_3	6.17 ± 2.02
CaO	26.01 ± 8.88
K_2O	0.42 ± 0.15
MgO	2.02 ± 0.87
Na_2O	1.08 ± 0.56
Fe_2O_3	3.96 ± 1.20
TiO_2	0.77 ± 0.21
P_2O_5	16.13 ± 5.33

Values are means $\pm$ s.d. for six samples.

Brushite, $\text{CaHPO}_4 \cdot 2\text{H}_2\text{O}$, and octacalcium phosphate, $\text{Ca}_8\text{H}(\text{PO}_4)_3 \cdot 2\text{H}_2\text{O}$, which are two crystalline forms known to occur in some calculus samples¹¹⁻¹³, were not discernible. It was not clear whether magnesium whitlockite, $(\text{Ca}, \text{Mg})_3(\text{PO}_4)_2$, another crystalline form found in some calculus, was present in any of the samples since certain major diffraction peaks for this substance fall in the same region as those characterising hydroxyapatite which was present. Insufficient sample sizes precluded fluoride analyses.

The presence of hydroxyapatite plus percentages of calcium and phosphorus similar to those reported for calculus¹¹⁻¹⁴ suggest, but do not confirm, that the accretions were originally laid down as dental calculus, not as lime or other simple calcium salts. Variations in the relative ratios of calcium, phosphorus, and magnesium indicate that some degradation has taken place on the calculus itself. This, together with the postmortem contamination, indicates that preservation of the material has been affected by environment, but not sufficiently to invalidate the basic conclusion.

The magnesium concentration in the samples was higher than that reported for modern calculus¹¹⁻¹⁴ but is compatible with the chewing of coca leaves since magnesium is a constituent of chlorophyll and a cofactor in some plant enzymes.

Since the lime was responsible for the deposits, the possibility that the people were chewing tobacco with lime cannot be ruled out altogether. Nevertheless, there is circumstantial evidence which argues in favour of coca use. In South America the historic distribution of chewing tobacco with lime was much more restricted than that of chewing coca with lime³. Furthermore, in the early historic period tobacco was used pre-eminently in ritualistic context whereas coca use was commonly secular and hedonic³. The huge calculus deposits imply habitual and, therefore, secular use. At Los Cerritos calculus deposits were more severe among males than females, suggesting that coca consumption by males was either more frequent or began at an earlier age.

Cultural evidence for the chewing of coca in coastal Ecuador appears as early as Valdivia times. Small ceramic lime pots have been found at Valdivian sites¹⁵, including Real Alto. In addition, a figurine recovered from the Valdivia-type site clearly depicts a quid in the cheek of a woman¹⁹.

Why, then, do the skeletons from Real Alto not show heavy deposits on the teeth? Presumably coca chewing expanded from a restricted, probably shamanistic, role in the Early Formative to a more popular, secular role in the Late Formative.

Since the climate of the Santa Elena Peninsula precludes the growing of coca, which thrives on the well-watered eastern slopes of the Andes, coca must have reached the coast by trade. Although there is evidence for trade routes as early as the Valdivia period^{16,17} commerce had become much more extensive by late Chorrera times. Paulsen¹⁸ presents evidence for the export of thorny oyster and conch shells from the Ecuadorian coast to the sierra beginning as early as 2800 BC. She concludes that the limited trade of the Early Formative had expanded to the Peruvian Andes by the first millennium BC. We propose that increased importation of coca to the south coast of Ecuador accompanied expanded exportation of mollusc shells to the sierra. In times of limited trade imported items would be at a premium and probably reserved for religious or curing purposes; expanded trade would make the exotic item, coca, available to a wider segment of the population for temporal use, in much the same manner as it is used by modern Andean peoples.

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High levels of cadmium in Atlantic seabirds and sea-skaters

THE possibility that cadmium may be an environmental pollutant has caused concern because it has toxic effects on many animal species including man, rat, rabbit, chicken, quail, and pigeon¹⁻⁴. The occurrence of cadmium in wildlife is not unusual and samples of marine species taken from around the British coast contain low levels (less than 0.5 mg per kg in fish and less than 2 mg per kg in most shellfish); however, levels of up to 93 mg per kg were present

in limpets, *Patellasp.*, collected from areas of natural mineralisation or industrial sources of cadmium⁵. Seabirds generally contain higher residues than marine invertebrates. For example, Anderlini *et al.*⁶ examined the amounts of nine metals in the livers of seven Antarctic and Pacific seabird species and noted that the maximum mean cadmium residue occurred in 10 ashly petrels *Oceanodroma homochroa* collected from their breeding grounds in California (mean residue was 53.2 ± 20.5 mg per kg). Parslow *et al.*⁷ found up to 22.3 mg per kg dry wt cadmium in livers of eight puffins *Fratercula arctica* collected live from various British breeding colonies. It was suggested that pelagic birds might contain higher residues than coastal living species because a single fulmar *Fulmarus glacialis* examined by them contained 159 mg per kg cadmium. Anderlini *et al.* concluded that there was a correlation between increased concentration of cadmium in birds and exposure to industrial influences. Parslow *et al.* did not attempt to explain the residues they found in puffins, but Bourne⁸ concluded that individual birds were becoming increasingly contaminated through feeding near areas of local pollution around the British coast. It is debatable whether cadmium should be regarded as a pollutant to seabirds. We report here the occurrence of cadmium residues in the tissues of apparently healthy, breeding seabirds which are considerably higher than any previously found. More important, we conclude that the cadmium has a natural rather than an industrial origin, since we have found high cadmium residues in a marine insect, *Halobates*, which is widely distributed in tropical regions far from sources of industrial cadmium. This insect is only one example of the sources from which birds obtain their cadmium residues.

Table 1 Cadmium residues in individual St Kilda seabirds

Species	Sex	Cadmium concentration (mg per kg dry wt)	
		Liver	Kidney
Fulmar <i>Fulmarus glacialis</i>	1* ♂	9.1	46.7
	2* ♀	50.1	184
	3 ♂	7.7	32.8
	4 ♀	49.4	240
Manx shearwater <i>Puffinus puffinus</i>	1 ♂	26.0	133
	2 ♀	39.9	231
	3 ♂	14.6	67.0
	4 ♂	15.6	113
Puffin <i>Fratercula arctica</i>	1 ♂	18.9	109
	2 ♀	14.1	75.1
	3 ♂	29.4	125
	4 ♂	57.0	68.5
Leach's petrel <i>Oceanodroma leucorhoa</i>	1 ♀	21.5	128
	2 ♂	20.6	80.0
	3 ♂	9.2	30.2
	4 ♂	14.4	52.9
Storm petrel <i>Hydrobates pelagicus</i>	1 ♂	20.8	37.7
	2 ♀	26.5	36.7
	3 ♂	2.4	14.6
	4 ♀	1.8	16.0
Razorbill <i>Alca torda</i>	1 ♂	1.4	18.2
	2 ♀		

Birds were killed by exsanguination and their tissues immediately frozen using solid CO₂ and kept below -20 °C until analysed for heavy metals. Metal analyses were performed by atomic absorption spectroscopy after tissues had been digested with concentrated nitric acid. Parentheses show birds known to be paired.

*Birds known to have an egg. Fulmars 3 and 4 were prospecting birds not yet ready to breed. All other subjects were thought to be breeding but this could not be confirmed due to inaccessibility of the nest site.

|| This bird had a shelled egg in the oviduct.

In June–July 1976 21 seabirds representing six species were collected, under licence, at a major breeding colony on St Kilda, a remote island group 80 km west of the Outer Hebrides, Scotland. All the animals were apparently healthy and unaffected by the metal levels they carried (Table 1). In all species, residues were higher in the kidney than in the liver, as in cadmium-contaminated humans¹. Cadmium residues were considerably higher in the pelagic species (shearwaters, puffins, and petrels) than in the razorbill, which feeds in the region of the continental shelf throughout the year^{9,10}. Since the pelagic species on St Kilda are unlikely to be exposed to industrial cadmium, it seemed possible

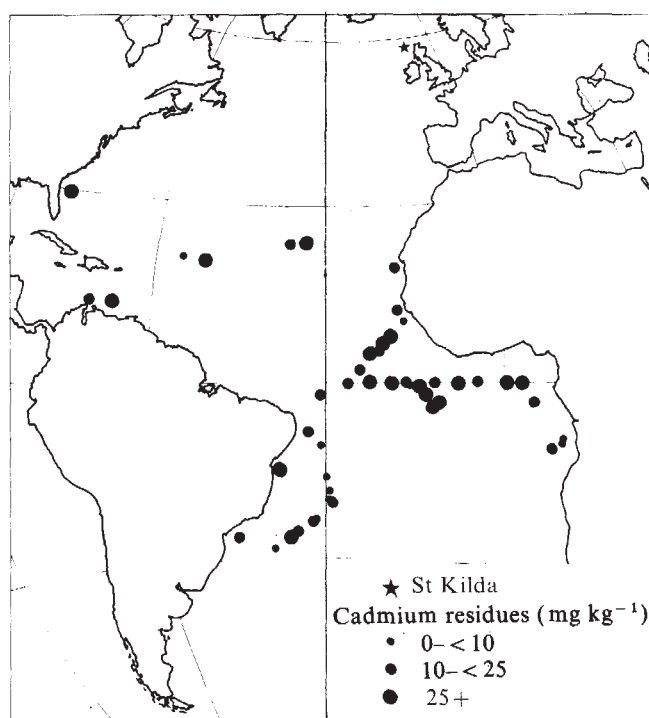
that there might be important sources of cadmium in the open ocean.

Leatherland *et al.*¹¹ examined a range of plankton organisms and their predators, although very small samples were involved. They found appreciable levels of cadmium in some species, the maximum being 13 mg per kg dry wt in the decapods *Systellaspis debilis* (four examined) and *Ophophorus* sp. (two examined). Plankton samples are usually of a variably mixed species composition and it is not easy to isolate a single species from a wide range of localities to check for geographical variations in cadmium content.

Sea-skaters of the genus *Halobates* (Gerridae: Heteroptera) are pelagic marine insects. They live at the sea surface and feed on zooplankton trapped at the air–sea interface. Relatively high cadmium contents have been detected in *Halobates* from various parts of the Pacific Ocean, and these may reflect correspondingly high cadmium levels in the waters where the samples were collected¹². It seems unlikely that the high cadmium levels detected in the sea-skaters resulted from atmospheric pollution, as no cadmium was detectable (< 5 mg per kg dry wt) in another gerrid, *Rheumatobates aestuarius*, collected from coastal mangrove lagoons in the Gulf of California, close to the area where *Halobates* was found at sea with very high cadmium levels (up to 200 mg per kg dry wt)¹². Since these insects are widely distributed in the oceans and single-species samples can be obtained over a large area, their cadmium contents can indicate the distribution of this heavy metal in the surface layers of the ocean.

Samples of *Halobates micans* were obtained from tropical areas of the Atlantic Ocean. These were rinsed of all foreign matter with acidified distilled water, digested in concentrated nitric acid and then analysed for cadmium using atomic absorption spectrophotometry. The distributions of residues in the samples were not uniform (Fig. 1) but there was no pattern suggesting pollution. The mean concentration in 111 samples was 22.7 ± 3.1 mg per kg dry wt, ranging from 0 to 309 mg per kg (in a sample from the Gulf of Guinea). In many instances several samples were collected at one

Fig. 1 Cadmium content of *Halobates micans* collected from 47 sites along survey routes in the Atlantic; the species is confined to warmer waters. In cases when more than one sample was collected from the same site the highest value has been plotted. St Kilda was the collecting locality for the seabirds analysed for cadmium (Table 1). Sources of *Halobates* samples and acknowledgments are given by Cheng¹².



site and in Fig. 1 maximum values are depicted; the mean for these was 30.5 ± 6.7 mg per kg ($n = 47$).

The concentration of cadmium in the ocean varies little from the depths to within 0.5 km of the surface layer, but declines rapidly near the surface, conceivably as a result of incorporation into living organisms¹³. Some of the areas where the higher cadmium residues occur, for example, off the coast of West Africa, have little industrial activity, but have upwelling ocean currents of cold water which bring to the surface nutrients, and possibly cadmium too. Areas of ocean upwelling are known to be important wintering locations for pelagic seabirds, because they are rich feeding grounds, and it is likely that the birds accumulate at least some of their residues in these places. The Manx shearwater spends the contranuptial season off the coast of South America, while the storm petrel winters off South Africa and Leach's petrel very widely in the tropics; puffins ringed on the western coast of Britain have been recovered south of the Straits of Gibraltar but the precise wintering grounds remain to be defined¹⁴. We cite the *Halobates* data as an indication of the widespread occurrence of cadmium in tropical waters. It is doubtful whether many seabirds take large quantities of *Halobates* though the phoenix petrel *Pterodroma alba* and blue-grey noddly tern *Procelsterna cerulea* have been proved to eat them in the Pacific^{15,16}. We might, therefore, expect these insects to be among the sources from which such birds as storm petrels could obtain cadmium.

We conclude that the high cadmium concentrations found in seabirds originate from natural sources, rather than from industrial activity, and that the birds, having evolved in an ecosystem containing cadmium, have developed, through natural selection, mechanisms which enable them to tolerate this metal. One such mechanism, now being investigated, involves production of a metal-binding protein which has been identified in the fulmar—it has many of the properties of metallothionein.

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Pectolytic anaerobic bacteria cause symptoms of cavity spot in carrots

CAVITY spot, a disease which causes considerable damage to the mature storage roots of carrots in several countries including the United Kingdom and the United States¹, has been reported to be caused by calcium deficiency, either actual or induced by excessive potassium levels in the soil². We have examined diseased carrots in

East Scotland since 1966 and have been unable to confirm the relationship with calcium levels. Evidence reported here indicates that pectolytic bacteria of the anaerobic genus *Clostridium* are the cause.

In the light of observations that the incidence of cavity spot was greatest in wet seasons on poorly drained land, and that more roots had lesions in waterlogged than in freely drained pots, the effect of restricting aeration to roots was investigated. Carrots were grown in plastic pots containing UC sand-peat compost (a mixture of three parts peat to one part coarse sand, formulated at the University of California) and when the crown diameters were greater than 20 mm, the compost surface was sealed with paraffin wax (melting point 55 °C). Roots of similar size were also lifted from the field, washed carefully and transplanted to similar pots. The pots were placed in trays containing 25 mm of water and kept in a controlled environment cabinet at 20 °C with an 18-h daylength of 12,000 lx. Analyses of gas composition in the compost (gas chromatogram, Pye model 106, MS 5A column, length 152.4 mm, oven temperature 100 °C) indicated that anoxic conditions developed within 48 h. After 5 or 6 d the wax was removed and the pots were returned to the glasshouse bench. Characteristic lesions were observed 3 weeks later. The proportion of roots affected was greatly increased when soil from a field outbreak was added to the roots before sealing. For example, the percentage of roots with lesions in an experiment in which all pots were sealed and supplemented with field soil or autoclaved field soil, or not supplemented, were 72, 28, and 24% respectively ($P < 0.001$ on contingency analysis).

Because there was evidence of a heat-labile factor in field soil and because no fungi or bacteria which were pathogenic on re-inoculation had been isolated by conventional techniques, isolations and subsequent inoculations were carried out under hydrogen in McIntosh and Fildes anaerobic jars fitted with palladium catalysts. Using a reducing medium with a pectate layer³, we isolated pectolytic bacteria at 25 °C from lesions formed on roots in sealed pots supplemented with field soil. Isolates were repeatedly sub-cultured from single colonies on streaked pectate plates and when grown in liquid basal medium anaerobically and inoculated to carrots, lesions identical to those caused by the soil supplement developed in sealed but not in open pots. The bacteria were recovered from these lesions by anaerobic culture.

Similar bacteria were obtained from several fields where cavity spot had occurred by inoculating carrot disks with about 1 g soil, and they were also recovered from some natural lesions incubated, like the disks, in anaerobic conditions. The colonies were cream coloured and the bacteria grew rapidly in anaerobic conditions at 25 °C producing a plaque in the pectate layer of up to 20 mm diameter in 2 d. Pectolysis on media and on carrot disks was still evident at 10 °C and no growth occurred on agar or on carrot disks in air. The bacteria were gram negative rods, 2.8–5.2 × 0.6–0.8 nm, which produced sub-terminal ovoid endospores, 1.4–2.4 × 0.8–1.0 nm. Gelatin was not liquified within 7 d at 25 °C. The organism was identified as a member of the genus *Clostridium*⁴, although results of further tests did not agree with those of any described species.

Cavity spot often occurs on poorly drained, compacted soils and it is most common after heavy rainfall in midsummer when the soil biomass and the high root population of carrots grown for canning may deplete oxygen more rapidly than it can be replaced by diffusion from the atmosphere. In these conditions it is suggested that the anaerobic bacteria described here may initiate cavity spot lesions. Pectolytic *Clostridium* spp. have been implicated in the rotting of potatoes in storage but they have not been associated previously with a field disease syndrome.

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Carcinogenic activity of hexachlorobenzene in hamsters

HEXACHLOROBENZENE (HCB), has been widely used as a fungicide, is a contaminant of various pesticides including DACTHAL and pentachloronitrobenzene, and is also a by-product in the manufacture of many chlorinated hydrocarbons (in the United States probably more than 2×10^6 pounds of HCB are produced annually¹). In Turkey, HCB was the causative agent in a severe outbreak of porphyria cutanea tarda symptomatica involving several thousand people between 1955 and 1959 (refs 2-4). In spite of the ban of HCB in 1959, symptoms of disease due to this toxic porphyria have persisted for a long time^{5,6}. The aim of present studies was to determine the chronic toxicity of HCB after prolonged oral administration and we report here that HCB is carcinogenic in hamsters.

Six-week-old Syrian golden hamsters from the Eppléy colony were used. They were fed *ad libitum* for life a diet containing 50, 100 and 200 parts per million (p.p.m.) HCB. The HCB was more than 99.5% pure (BDH, England). Dosage regime and experimental design are shown in Table 1. At 50 weeks of age, 71% of the treated animals were alive, a survival rate

Table 1 Dosage regime for HCB in hamsters

Group	No. of animals		Concentration of HCB in diet (p.p.m.)	Duration of treatment	Calculated daily intake (mg kg ⁻¹ d ⁻¹)
	F	M			
Control	40	40	0	Lifespan	0
Low	30	30	50	Lifespan	4
Medium	30	30	100	Lifespan	8
High	60	59	200	Lifespan	16

comparable with that of the controls. After 70 weeks, a shorter lifespan occurred in both females and males treated at the highest dose level of HCB; compared with the controls, males treated at 200 p.p.m. HCB showed a marked weight reduction. Table 2 gives cumulative data on the tumour incidence at each dose level. The percentage of tumour-bearing animals (TBA) varied, with controls showing a 10% incidence and hamsters treated at 200 p.p.m. HCB, a 92% average incidence. There was, therefore, an obvious difference relative to treatment. The average number of tumours per hamster and the percentage of hamsters with more than one tumour also showed a dose-response relationship.

All 15 thyroid tumours found were alveolar adenomas and were present only in treated animals. There was a significant increase ($P < 0.05$) in occurrence of these tumours in males treated at 200 p.p.m. HCB, when compared with male controls.

No hepatomas were seen in the control group. In the HCB-treated animals, the incidence increased from 46% in the group treated at 50 p.p.m. to 85% in hamsters receiving 200 p.p.m. HCB. No hepatoma metastases were found. The first hepatoma (with multiple nodules, the largest in diameter being 7 mm) occurred in one female hamster that died after 18 weeks of treatment.

Liver haemangioendotheliomas were also observed only in the HCB-treated group. The highest and significant incidence occurred in the group treated at 200 p.p.m. HCB, consisting of 11.6% of females and 35% of the males. Three of the haemangioendotheliomas in these animals metastasised. A few haemangioendotheliomas were observed in the spleen.

Among other tumours, an increased incidence of adrenal neoplasms occurred; however, no clear dose-response relationship was shown, nor was there a significant difference between the groups.

The results indicate the carcinogenicity of HCB. In the case of other known carcinogens, such as dimethylnitrosamine, vinyl chloride, 2-acetylaminofluorene and 2-naphthylamine, there is a wide spectrum of carcinogenic activity in different tissues and species, including the hamster. In our present experiment, doses of HCB ranging from 50 to 200 p.p.m. resulted in a significant induction of hepatomas, haemangioendotheliomas and thyroid adenomas. Further, an increased number of tumour-bearing animals and of tumours per animal was noted, as were a shortened lifespan and a reduced latency period for onset of liver tumours. These effects may indicate that HCB behaves like certain carcinogens shown to have multipotential activity.

A purely quantitative approach indicates that an intake of 4-16 mg kg⁻¹ d⁻¹ of HCB in our hamsters was within the range of the estimated quantities accidentally consumed by the Turkish people for several months at a time, resulting in severe toxic porphyria⁴.

In a study conducted in Louisiana, in 86 persons who resided in an HCB-contaminated area, the mean HCB concentration in plasma samples was 0.0036 p.p.m. (ref. 7). In yet another investigation, farm workers occupationally exposed to HCB-contaminated DACTHAL had blood HCB concentrations averaging 0.040 p.p.m. but there was no evidence of porphyria in this group⁸. It must, however, be stressed that with Burns' two cross-sectional epidemiological studies^{7,8}, it would have been very difficult to demonstrate the carcinogenicity of HCB.

The extrapolation of the present experimental results to man is not possible without considering all available data on the biological effects of HCB. In this sense, we believe there is urgent need to obtain epidemiological information to evaluate the relevance of the experimental studies.

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Table 2 Tumour incidence in hamsters given HCB

Group	Effective no.	TBA no.	%	No. of tumours per hamster		More than one tumour		Thyroid		Hepatoma		Haemangioendotheliomas Liver		Spleen		Other	
				No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Control	39 F	5	12.8	5	0.13	0	0	0	0	0	0	0	0	1	2.5	4	10.2
	40 M	3	7.5	3	0.08	0	0	0	0	0	0	0	0	0	0	3	7.5
HCB50	30 F	16	53.3	21	0.70	4	13.3	2	6.6	14	46.6	0	0	0	0	5	16.6
	30 M	18	60.0	27	0.90	8	26.6	0	0	14	46.6	1	3.3	1	3.3	11	36.6
HCB100	30 F	18	60.0	32	1.06	11	36.6	1	3.3	17	56.6	2	6.6	3	10.0	9	30.0
	30 M	27	90.0	45	1.50	14	46.6	1	3.3	26	86.6	6	20.0	3	10.0	9	30.0
HCB200	60 F	52	86.6	73	1.21	15	25.0	3	5.0	51	85.0	7	11.6	4	6.6	8	13.3
	57 M	56	98.2	87	1.52	27	47.3	8	14.0	49	85.9	20	35.0	4	7.0	6	10.5

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Effect of aspirin administration on retinoic acid toxicity in mice

THIS study was designed to provide an indirect assessment of the possible role of prostaglandins in retinoid intoxication. If the symptoms of retinoid toxicity are mediated by prostaglandins, an inhibitor of prostaglandin synthesis should ameliorate the symptoms of sublethal intoxication and perhaps even increase the lifespan of mice treated with lethal doses of retinoids. The observations reported here indicate that aspirin can antagonise the toxic effects of retinoic acid.

Sporn *et al.*^{1,2} have presented information on the biological activity of a number of retinoids (vitamin A analogues). Retinoids seem to influence differentiation of epithelial cells in several tissues to the extent that in certain test systems, they prevent or reverse malignant transformation of epithelial cells exposed to known carcinogens¹. This activity holds promise for the 'chemoprevention'² of cancer in man. Since chemoprevention will involve use of a drug by individuals who have a greater than average chance of developing cancer but who may be healthy otherwise, the acceptability of retinoids may depend on their toxicity. Excessive doses of retinol or all-trans-retinoic acid (RA) produce a hypervitaminosis A syndrome in man and experimental animals³⁻⁶. Hixson and Denine⁶ suggested that this syndrome might be more appropriately referred to as retinoid intoxication, since many of the retinoids exhibit similar side effects, including headache, vomiting, pruritus, skeletal pain, and bone resorption^{3-5,7}.

The mechanism of retinoid toxicity has been open to question^{1,2}, but the syndrome suggests a possible mediator. Headache^{8,9}, vomiting⁹, pruritus⁸, skeletal pain^{10,11} and bone resorption¹²⁻¹⁴ are common symptoms of retinoid intoxication and prostaglandin activity. Hypothetically, the symptoms of retinoid intoxication may be mediated by prostaglandin production. As a preliminary test of this hypothesis, we have treated mice with a combination of aspirin and toxic doses of RA. Vane⁸ demonstrated that aspirin inhibits prostaglandin production, and subsequent studies have confirmed his conclusions^{15,16}. Since bone thinning (resorption) and occurrence of fractures provide a dose-dependent indication of retinoid intoxication in mice⁶, we have used this endpoint and the incidence of mortality to demonstrate that aspirin protects mice from retinoid intoxication.

To produce retinoid intoxication in mice, RA (30 mg kg⁻¹) was administered intraperitoneally (i.p.) daily for 21 d

(Q1D×21). This dose approximated the LD₅₀ obtained previously with this schedule in our laboratories^{6,17}. RA was suspended in an aqueous solution of 8% Cremophor EL (Sigma) and 10% propylene glycol; these suspensions were protected from light. Aspirin (ASA) was suspended in a mixture of physiological saline and 0.3% hydroxypropyl cellulose. Sixty adult Swiss mice weighing approximately 24 g (range: 21-27 g) were distributed into three experimental groups of 20 mice each (10 male, 10 female). Drugs were administered according to individual body weight as follows: RA only, 30 mg kg⁻¹ i.p., Q1D×21; ASA only, 150 mg kg⁻¹ by mouth (p.o.), Q1D×21; RA 30 mg kg⁻¹ i.p. plus ASA 150 mg kg⁻¹ p.o., Q1D×21. Mice were weighed twice weekly and X-rayed on treatment days 1, 8, 15, and on the day after the last treatment. X-ray films were evaluated weekly to determine the number of fractured bones per mouse. During the treatment period, including the day after last treatment, deaths per total mice (group) were: 10/20 (RA), 1/20 (ASA), and 0/20 (RA+ASA). This experiment was repeated: 60 mice weighing approximately 26 g (range: 22-30 g) were distributed and treated as before, except that these mice were not X-rayed on treatment day 1. In this experiment, deaths per total mice (group) were: 7/20 (RA), 0/20 (ASA), and 3/20 (RA+ASA).

The frequency of deaths and the frequency of fractures among survivors on days 8, 15, and one day after last treatment were arranged as a contingency table for each experiment. Since no fractures occurred in ASA-treated mice, they were excluded. Chi-square analysis¹⁸ indicated that the data from the two experiments were sufficiently homogeneous to justify pooling them. The pooled data (Table 1) were then tested for statistical significance¹⁸. The difference in mortality between the RA group (17/40) and the RA+ASA group (3/40) was significant ($P < 0.001$) on the day after last treatment. On day 15, the number of mice with three or more fractures was significantly greater in the RA group than in the combination group ($P < 0.05$); no other significant differences were observed and there seemed to be no sex differences. Differences were apparent between the general condition of mice in the RA group and the RA+ASA group on the day after last treatment. Loss of body weight, for example, is characteristic of sublethal retinoid intoxication.

Data presented in Table 1 suggest that ASA treatment either delayed or antagonised the development of sublethal retinoid toxicity. Although no differences were detected in the number of fractures after three weeks of drug delivery, the reduced mortality in the RA+ASA-treated mice strengthens the suggestion of benefit from ASA treatment. Agents other than ASA are more potent inhibitors of prostaglandin synthesis¹⁵; and if prostaglandins do play a part in retinoid intoxication, the protection indicated by these results can probably be improved with optimal treatment.

Although more direct support of our hypothesis is required, a question worthy of early consideration is how prostaglandins might fit into a mechanistic scheme for retinoid intoxication. Mallia *et al.*¹⁹ have concluded that retinol toxicity occurs in rats when the binding capacity of plasma retinol-binding protein is exceeded. Plasma concentrations of free retinol and retinyl esters then become elevated. These results have also been demonstrated in humans²⁰. Similarly, saturation of intracellular or extracellular proteins that bind retinoids would result in higher concentrations of free retinoids. Free retinol^{19,20} and free retinoids^{21,22} destabilise lysosomal membranes. The subsequent release of lysosomal enzymes, particularly phospholipase A, induces prostaglandin synthesis²³. Destabilisation of lysosomal membranes and release of lysosomal enzymes have been invoked to explain the local elevation of prostaglandin concentrations following

Table 1 Effects of retinoic acid alone and in combination with aspirin on Swiss mice

Day	Group	0	No. of fractures per survivor			No. of deaths per total
			1	2	3+	
8	RA	22/40	13/40	5/40	0/40	0/40
	RA + ASA	16/40	20/40	3/40	1/40	0/40
15	RA	7/36	10/36	8/36	11/36*	4/40
	RA + ASA	9/38	15/38	10/38	4/38*	2/40
22 (Day after last treatment)	RA	5/23	4/23	3/23	11/23	17/40†
	RA + ASA	5/37	7/37	13/37	12/37	3/40†

* The number of animals with three or more fractures is significantly lower in the RA + ASA group ($P < 0.05$).

† The number of deaths is significantly lower in the RA + ASA group ($P < 0.001$).

application of RA to guinea pig skin²⁴. Our observations suggest that systemic administration of RA has a similar effect. Rational control of retinoid intoxication will have an important influence on the development of retinoids for human use.

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L-Dopa methyl ester as a new antitumour agent

L-DOPA (L-3,4-dihydroxyphenylalanine) is extensively used in man for the treatment of Parkinson's disease, and it can also inhibit selectively the growth of pigmented human melanoma cells *in vitro*¹. Demonstration of *in vivo* antitumour activity, however, has been lacking except in the special case of hormonally responsive mammary carcinoma² where the antitumour effect is thought to be mediated indirectly. Since *in vitro* cytotoxicity was not observed until concentrations of 600–1,200 $\mu\text{g ml}^{-1}$ were attained the use of the more soluble methyl ester derivative was proposed as a method for delivery of cytotoxic doses. *In vivo* antitumour activity of L-dopa methyl ester is reported here in the following tumour systems: L1210

lymphocytic leukaemia; P388 lymphocytic leukaemia and B-16 melanoma.

The results of *in vivo* antitumour experiments are summarised in Table 1. L-Dopa itself, because of insolubility, could not be administered to mice at doses greater than 100 mg kg^{-1} and did not show therapeutic activity at this dose. The methyl ester at the maximally tolerated dose of 600 mg kg^{-1} , however, exhibits activity in each of the test systems and is relatively

Table 1 *In vivo* antitumour activity of L-dopa methyl ester against L1210 and P388 murine leukaemias and B-16 melanoma

Drug	Dose (mg kg^{-1})	% Increase lifespan (ILS)*		
		L1210	P388	B-16
L-dopa methyl ester	500	+33	+46	+22
	600	+33	+27	+34(+50)‡
Ro4-4602	200	+22	+10	0
MK 486	100	+10†	+0	0
Ro4-4602 and L-dopa methyl ester	200 + 800			+25
	200 + 1,000	+44	+45	+30
	200 + 1,250	+55	+36	
MK 486 and L-dopa methyl ester	100 + 1,000	+55	+70	

L-Dopa and Ro4-4602 were gifts from the Hoffman LaRoche Company, Nutley, New Jersey; MK 486 was from the Merck, Sharpe and Dohme Co., West Point, Pennsylvania; L-dopa methyl ester was from the Sigma Chemical Company, St Louis, Missouri. Antitumour activity of each compound was determined by measuring the median ILS of mice given intraperitoneal (i.p.) inoculations of either 1×10^6 P388, 1×10^5 L1210 lymphocytic leukaemia cells or 0.5 ml of a 1:10 homogenate of B-16 melanoma tumour. The assay procedures followed the standard National Cancer Institute protocols³ for these tumour systems, with treatment beginning on the first day after tumour inoculation and continuing daily for 7 d with L1210 and P388 and 12 d for B-16 tumour. Agents were given i.p. in saline. Ro4-4602 was given 1 h before treatment with ester. C57Bl $\times$ DBA/2 F₁ male mice (Jackson Laboratory, Bar Harbor, Maine) were used throughout. Percentage ILS was calculated according to the equation: % ILS = $100 \times (t/c - 1)$ where t is the median survival time (in d) of the treatment group and c is the median survival time of the control group.

*Values are significant at $P < 0.001$ unless otherwise noted.

†NS, Not significant.

‡Administered daily for 24 d.

active against the B-16 melanoma, which is considered to be highly refractory to conventional agents. The combination of L-dopa methyl ester with the inhibitors of dopa decarboxylase benserazide (Ro4-4602) and carbidopa (MK 486) (refs 3, 4) results in a marked increase in both the antitumour effect and the maximally tolerated dose.

Table 2 summarises the effects of L-dopa methyl ester on thymidine, uridine and leucine incorporation in the S-91 Cloudman melanoma and L929 mouse fibroblast *in vitro*. There is a selective and profound inhibition of DNA synthesis as measured

during drug exposure, with relatively greater inhibition in the melanoma cells. Uridine and leucine incorporation are largely unaffected. The greater inhibition observed in the pigmented line as well as the activity observed against B-16 melanoma may be related in part to the role of L-dopa as a biochemical intermediate in the formation of the pigment, melanin¹.

Although the precise cellular target of L-dopa methyl ester is not known, the pharmacological factors important to *in vivo* activity may be inferred. The acute toxic effects of L-dopa are mediated by its conversion to dopamine by the enzyme L-aromatic amino acid decarboxylase. At the high doses of L-dopa administered, a hypercatecholamine-type state was observed. The animals became agitated and tremulous and usually died with 1–2 h after administration. These acute toxic effects of L-dopa are probably mediated by its conversion to dopamine by the enzyme, L-aromatic amino acid decarboxylase (dopa decarboxylase). The striking increase in both the therapeutic

through inhibition of DNA synthesis. Extensive experience with both L-dopa and dopa decarboxylase inhibitors in human disease should permit rapid evaluation of their potential clinical significance.

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Table 2 Inhibition of macromolecule biosynthesis by L-dopa and L-dopa methyl ester in S-91 Cloudman melanoma and mouse fibroblast L929 *in vitro*

Cell line	L-Dopa	DNA	RNA	Protein
	Concentration ($\mu\text{g ml}^{-1}$)			
S-91	600	49 $\pm$ 4	15 $\pm$ 6	10 $\pm$ 5
	1,200	70 $\pm$ 5	30 $\pm$ 5	16 $\pm$ 8
L929	600	41 $\pm$ 4	21 $\pm$ 5	34 $\pm$ 8
	1,200	49 $\pm$ 5	22 $\pm$ 6	32 $\pm$ 5
L-dopa methyl ester				
S-91	600	96 $\pm$ 1	44 $\pm$ 12	27 $\pm$ 7
	1,200	98 $\pm$ 1	65 $\pm$ 5	47 $\pm$ 10
L929	600	74 $\pm$ 2	44 $\pm$ 5	52 $\pm$ 8
	1,200	84 $\pm$ 3	67 $\pm$ 11	76 $\pm$ 8

Values represent % inhibition and are mean $\pm$ s.e.m. for triplicate determinations. The origin and maintenance of cell lines has been described in detail previously. Cells were grown in McCoy's 5A medium supplemented with 15% foetal calf serum, 100 units ml^{-1} of penicillin and 100 $\mu\text{g ml}^{-1}$ of streptomycin¹. Cells were plated in Linbro multi-well tissue culture trays and after 48 h they were in the log phase of growth. Medium was then aspirated, the monolayer washed and 1 ml of serum-free medium containing 2 $\mu\text{Ci ml}^{-1}$ of either ³H-thymidine (specific activity 2 Ci mmol^{-1}), ³H-5-uridine (specific activity 25 Ci mmol^{-1}), or ³H-leucine (specific activity 41 Ci mmol^{-1}) (New England Nuclear, Boston, Massachusetts) was added. After 60 min at 37 °C medium was removed, cells were washed once with saline and 1 ml of 10% trichloroacetic acid was added. The precipitate was washed three times with 0.9% sodium chloride and 0.5 ml of 1.0 M KOH added. Precipitate containing RNA, DNA and protein was then digested at 37 °C for 4 h and an aliquot added to scintillation vials containing Aquasol (New England Nuclear).

effect and maximally tolerated dose observed by pretreatment with inhibitors of this enzyme, may be related to the resulting elevated plasma levels of L-dopa and decreased levels of dopamine⁵. Alternatively, since Ro4-4602, an analogue of L-dopa, exhibited significant antitumour activity in L1210 and P388 systems, ($P < 0.001$), the increase in activity of this combination may be partially the result of an additive antitumour effect.

An intriguing possibility concerning the mechanism of action of L-dopa methyl ester and Ro4-4602 may be proposed. It is well known that L-dopa can combine with cysteine to yield 5-cysteinyldopa, a metabolite found in large amounts in the urine of patients with metastatic melanoma⁷. Livingston *et al.* have demonstrated⁸ that a large number of lymphoid and myeloid tumour lines, including L1210 and P388, are unable to grow in tissue culture in the absence of pre-formed L-cysteine⁸. It is possible, therefore, that L-dopa methyl ester and its analogue Ro4-4602 function as sulphhydryl scavengers, depriving tumours of essential growth factors.

L-Dopa methyl ester is a novel and potent antitumour agent in experimental tumour systems. It seems to act primarily

Dopamine agonists induce recovery from surgically-induced septal rage

ALTHOUGH recovery of function following brain damage has long been known to occur, the mechanisms involved are not completely understood¹. Of relevance to this problem are reports of pharmacological facilitation of recovery from brain damage in humans² and from experimentally-induced nervous system lesions in animals^{3–9}. We have previously reported that L-dopa injections produce a dramatic and apparently permanent abolition of the hyper-reactivity or rage syndrome that results from surgical damage to the septal nuclei of the rat forebrain¹⁰. Independent confirmation of this finding has also appeared¹¹. We report here that other dopamine agonists share this ability to attenuate or abolish the septal rage syndrome (SRS).

Individually housed male Long-Evans hooded rats (280–320 g) were anaesthetised with sodium thiopental and subjected to bilateral radio frequency lesions of the septal nuclei, using standard surgical and stereotaxic techniques. These lesions destroyed the medial and lateral septal nuclei and damaged the adjacent caudate nuclei, nucleus accumbens septi, and striatum terminalis.

After recovery from the anaesthesia, approximately 60% of the lesioned animals exhibited a clear and persistent SRS, consisting of explosive reactivity to visual, tactual and auditory stimulation, coupled with pronounced biting, attack, fleeing, muscle tension and muricide¹². These hyper-irritable animals were then returned to their home cages; and, on the next day, rated for SRS hyperirritability¹³ in special observation chambers. Each animal was then given an intraperitoneal injection of one of the following: L-dopa (100, 60, 40, 30 or 10 mg per kg body weight), apomorphine (20, 15, 10 or 5 mg per kg), pibedil (300 or 200 mg per kg), amphetamine (4 or 2 mg per kg), methohexital (40 mg per kg), or saline. Each animal was returned to its home cage, and then placed in the observation chamber and rated for hyperirritability at 0.5, 1, 1.5, 2, 3, 4, 24, 48, 72, 96, and 120 h post-injection. All ratings of drug-injected animals were done blind. The methohexital treated animals were additionally rated at 0.25 and 0.75 h post-injection, and the apomorphine animals were also rated at 5 and 6 h. A few animals from some groups were also rated at times beyond 120 h (up to 1 month after injection). In this particular preparation, competing stereotypic behaviours were not observed for any of the doses of the catecholaminergic agonists used. Statistical analyses of changes in hyperirritability were performed as appropriate using the Friedman non-parametric analysis of variance (χ^2), the Wilcoxon matched-pairs signed-ranks test (T), and the Mann-Whitney rank test for independent samples (U).

Saline-injected animals (Fig. 1f) exhibited a constant level of irritability for the first 24 h, followed by gradual diminution over

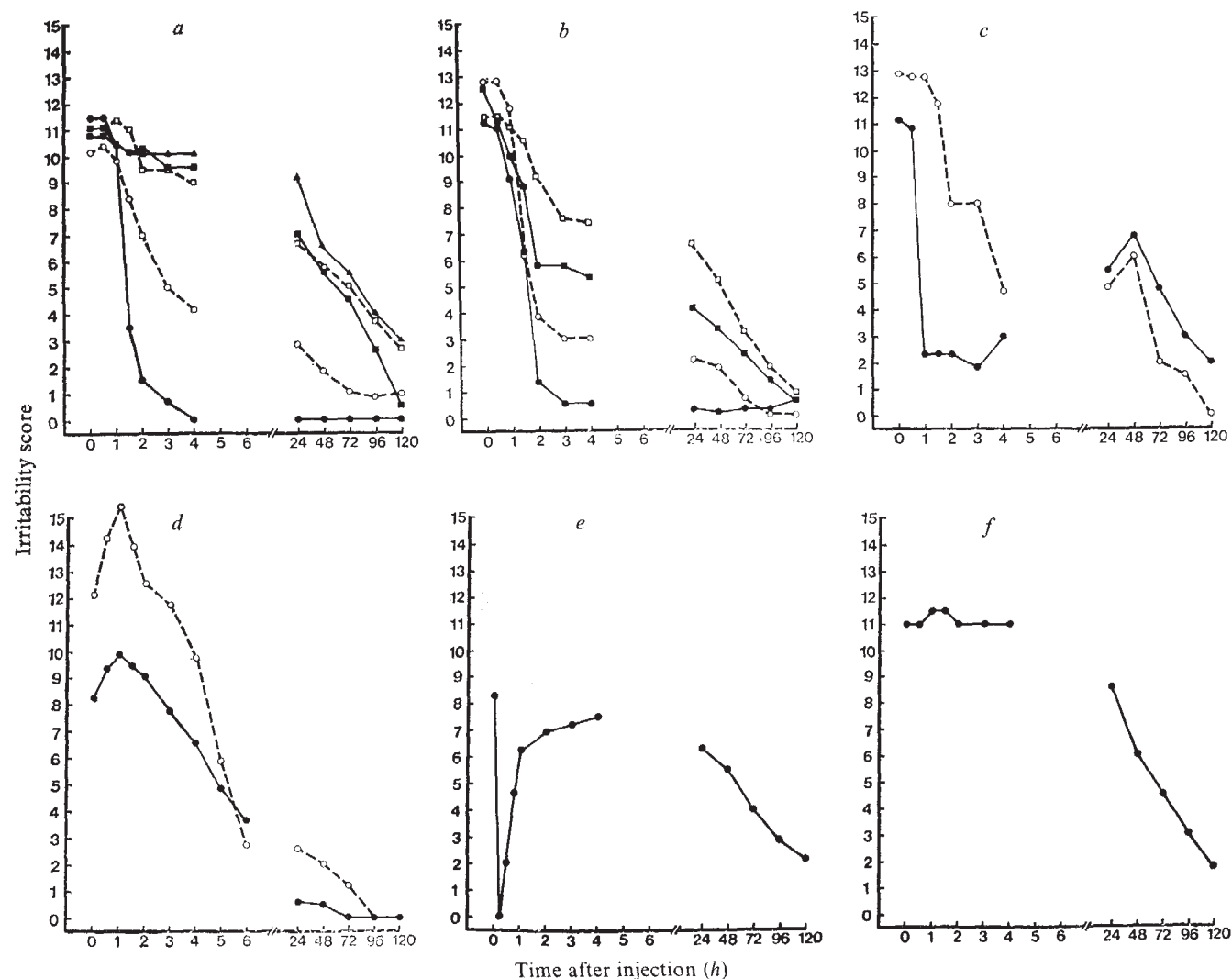


Fig. 1 Post-injection changes in SRS brain lesion symptoms ('irritability') following single administrations of *a*, L-dopa: ●, 100 mg per kg body weight, $n = 8$; ○, 60 mg per kg, $n = 5$; ■, 40 mg per kg, $n = 3$; □, 30 mg per kg, $n = 4$; ▲, 10 mg per kg, $n = 4$. *b*, Apomorphine: ●, 20 mg per kg, $n = 12$; ○, 15 mg per kg, $n = 5$; ■, 10 mg per kg, $n = 6$; □, 5 mg per kg, $n = 5$. *c*, Piribedil: ●, 300 mg per kg, $n = 7$; ○, 200 mg per kg, $n = 3$. *d*, Amphetamine: ●, 4 mg per kg, $n = 10$; ○, 2 mg per kg, $n = 6$. *e*, Methohexital, 40 mg per kg, $n = 14$. *f*, Saline, $n = 10$.

the ensuing 5 d ($\chi_r^2 = 47.8$, $P < 0.001$), similar to non-injected animals. In contrast, animals given L-dopa (100 mg per kg) showed a dramatic and prompt return (Fig. 1*a*) to pre-operative levels of irritability (for the 0–4 h post-injection period, $\chi_r^2 = 43.8$, $P < 0.001$). Latency for a measurable effect was 90 min ($T = 0$, $P < 0.01$), and by 120 min after injection, the animals were indistinguishable from non-lesioned controls (the difference between the 100 mg per kg L-dopa animals and the saline controls also reaching significance by 120 min after injection; $U = 1.5$, $P < 0.001$). The L-dopa animals showed normal righting, placing and balance reflexes, and gave no appearance of being sedated; they continued to eat and drink, and remained normally responsive to environmental stimuli. The L-dopa abolition of lesion-induced SRS symptoms was also noteworthy for its permanence; none of the 100 mg per kg treated animals showed return of hyperaffective behaviour when examined up to 1 month post-injection. At 60 mg per kg L-dopa, a less marked, but still prompt and clear, attenuation of lesion-induced symptoms occurred ($\chi_r^2 = 19.8$, $P < 0.01$).

Animals given apomorphine also showed a drug-induced SRS amelioration (Fig. 1*b*). Apomorphine at 20 mg per kg produced complete and permanent abolition of lesion-induced symptoms within 120 min ($\chi_r^2 = 35.4$, $P < 0.001$); lower doses produced less effect on the time course of recovery (Fig. 1*b*). As with L-dopa, the animals were not merely sedated, and the drug-induced behavioural change was permanent.

Piribedil and D,L-amphetamine produced differing effects on the time course of SRS recovery (Fig. 1*c, d*). Piribedil produced the same initial SRS amelioration during the first 4 h as L-dopa and apomorphine (χ_r^2 for 200 mg per kg piribedil = 15.1, $P < 0.01$; χ_r^2 for 300 mg per kg piribedil = 26.7, $P < 0.001$). But, this was followed by subsequent SRS increase at 24 and 48 h (most noticeable in the 300 mg per kg animals, which showed the most SRS attenuation during the first 4 h ($\chi_r^2 = 9.3$, $P < 0.05$)). The irritability of the piribedil-injected rats then attenuated over the next 4 d (χ_r^2 for 200 mg per kg = 8.5, $P < 0.01$; χ_r^2 for 300 mg per kg = 13.0, $P < 0.01$) at the same rate observed in untreated controls. Amphetamine produced an initial SRS increase during the first 90 min (only the 2 mg per kg animals being significant; $\chi_r^2 = 9.25$, $P < 0.01$), followed by a rapid and permanent SRS abolition (χ_r^2 for 2 mg per kg = 44.4, $P < 0.001$; χ_r^2 for 4 mg per kg = 43.6, $P < 0.001$).

In marked contrast to the L-dopa, apomorphine, piribedil or amphetamine effects, the short-acting barbiturate methohexital produced immediate sedation ($T = 0$, $P < 0.001$), followed by rapid return to full, lesion-induced SRS irritability ($\chi_r^2 = 29.1$, $P < 0.001$), which then attenuated over the next 5 d ($\chi_r^2 = 27.8$, $P < 0.001$) at a rate similar to control animals (Fig. 1*e*). Essentially, methohexital produced a brief and profound sedation, but no effect on the time course of recovery from the lesion. Thus, the action of L-dopa and apomorphine cannot be ascribed to the effect of handling during transient tranquillisation.

Apomorphine seems relatively selective in its dopamine receptor stimulating properties^{14,15}, so activation of dopamine receptors seems sufficient to induce neuronal processes leading to recovery from septal damage. The initial SRS exacerbation under amphetamine may reflect a noradrenergic effect (preliminary data from SRS animals given imipramine, which also seems to intensify the SRS symptoms, seem confirmatory); the biphasic response to pibedil may reflect an action on presynaptic release of dopamine at brain sites distinct from the sites wherein apomorphine and L-dopa exert their effects¹⁶.

Our data contrast with reports of pharmacological diminution of SRS symptoms by neuroleptic agents, barbiturates, physostigmine, chlordiazepoxide and non-narcotic analgesics¹⁷⁻²⁰. In these cases, the effects were transient and most closely resemble the present methohexital effect (albeit with less brief and profound behavioural inhibition). There are also reports of sedative and/or excitatory effects of L-dopa and related compounds on normal or brain-damaged animals, but these effects, too, are transient and reversible. The only previous report of permanent SRS abolition is that of Dominguez and Longo⁹, using *p*-chlorophenylalanine (pCPA). These data have been interpreted to implicate a serotonergic role in SRS recovery⁹, but we suggest that they may support a catecholaminergic (possibly dopaminergic) role in SRS recovery, since the time course of the action of pCPA action on the SRS is more consistent with an action on catecholaminergic systems²¹⁻²³.

The time course that we find for dopamine agonist-induced SRS recovery is only a few hours. Most neurological or behavioural processes invoked as explanations for recovery from brain damage have time courses measured usually in weeks, for example neural reorganisation, vicarious function, denervation supersensitivity, axonal sprouting and regrowth, and behavioural substitution¹. Changes in blood-brain barrier permeability²⁴ and diaschitic alterations in brain function^{2,25} may have shorter time courses, however. We are unable to find any alteration in blood-brain barrier permeability to noradrenaline in septally lesioned animals displaying SRS symptoms (uptake of intravenous ³H-noradrenaline into brain tissue was studied in six SRS, six septally-lesioned non-irritable, and six sham-operated rats 24 h after surgery; no differences between groups were found). The possibility of a diaschitic process remains—dopaminergically induced SRS recovery may involve the return of function in transiently dysfunctional regions either adjacent to or neurally connected to the lesioned area. Possible regions of transient dysfunction might include the fibres and nuclei of the dopamine-rich mesolimbic forebrain system^{26,27}, for example, the nucleus accumbens adjacent to the septal nuclei, and the olfactory tubercle and anterior amygdala, reciprocally connected to the septum²⁸. The mechanism by which dopaminergic stimulation might hasten recovery of function in these areas remains to be determined.

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Viable chimaeras produced from normal and parthenogenetic mouse embryos

PARTHENOGENESIS occurs spontaneously in about 10% of ovulated eggs of inbred strain LT/Sv mice^{1,2} and is experimentally inducible in other strains by various physical and chemical agents (see ref. 3 for review). The development of most parthenotes seems normal up to the expanded blastocyst stage⁴. They implant in the uterus, but, for reasons unknown, are resorbed within a few days. Although they rarely survive to 8 d of gestation when they develop somites, heart muscle, amnion, and neuroepithelium, Kaufman *et al.*⁵ obtained two embryos with 25 somites by transferring parthenogenetic blastocysts to the uteri of ovariectomised females treated with exogenous hormones. Even though parthenogenetic embryos do not survive to birth, their cells contain genetic information that permits prolonged survival. If two-cell parthenotes are cultured to the blastocyst stage and then grafted to extrauterine sites such as the testis or kidney, they may survive as teratomas composed of several types of tissues and undifferentiated embryonic cells (refs 1, 6 and L.C.S. and D.S.V., unpublished). Furthermore, strain LT/Sv spontaneous ovarian teratomas contain numerous differentiated tissues composed of parthenogenetically derived cells. The questions remains—why do parthenotes not survive at the organismic level *in utero*? Eicher and Hoppe⁷ used experimental chimaeras composed of normal and abnormal embryos to transmit a recessive X-linked lethal mutation, and we considered the possibility that parthenogenetic embryonic cells might also be rescued if combined with normal embryonic cells. Here we present evidence of production of at least two viable chimaeras between normal and parthenogenetic embryos.

Parthenogenetic eight-cell embryos were obtained from F₁ hybrid females produced by mating females of the LT/Sv strain to males of the incipient recombinant inbred strain LTXBJ (L.C.S. and D.S.V., unpublished). (The progenitors of LTXBJ were strains LT/Sv and C57BL/6J. Strain LTXBJ had been inbred for 11 generations of brother × sister matings when the experiments were initiated.) Nearly all of the females of the LTXBJ recombinant strain have bilateral ovarian teratomas at 3 months of age. These teratomas are derived from parthenogenetically activated ovarian eggs. About 30% of their ovulated eggs undergo spontaneous parthenogenetic development. (LT/Sv × LTXBJ)F₁ hybrid females have the same high incidence of spontaneous parthenogenesis as LTXBJ females.

Inbred strain LT/Sv is homozygous for the alleles *a*, *B^{et}*, *C*, and *Gpi-1^a* at the agouti, brown, albino, and glucose-phosphate isomerase-1 loci, respectively. LTXBJ is homozygous for the

alleles *a*, *B*, *C*, and *Gpi-1^b*. Although parthenogenetic embryos from the LT/Sv strain are diploid (P. C. Hoppe, personal communication), it is not known whether they result from initiation of mitosis in primary oocytes or secondary oocytes (lack of the second meiotic division), or diploidisation of haploid ootids. Regardless of their origin the parthenogenetic embryos from the (LT/Sv × LTXBJ)_F females would appear *a/a C/C*. Depending on the nature of their origin, however, their genotypes for the brown and glucose-phosphate-isomerase-1 loci could be homozygous (for either allele) or heterozygous. Interpreting the origin of parthenogenetic cells in chimaeras was further complicated because we combined two parthenogenetic embryos per single normal embryo to form each chimaera. The two parthenotes could have different genotypes.

The normal eight-cell embryos were derived from eggs of albino 129/Sv (homozygous *A^w*, *B*, *c*, *Gpi-1^a*) females fertilised by sperm from A/HeJ (homozygous *a*, *b*, *c*, *Gpi-1^b*) males. Thus, these *F*₁ embryos were genotypically *A^w/a, B/b, c/c, Gpi-1^a/Gpi-1^b*.

Experimental chimaeras were produced by aggregating eight-cell embryos *in vitro*⁸. After removal of the zona pellucida with Pronase, one normal and two parthenogenetic morulae were combined in Whitten's medium⁹ and cultured overnight. The aggregated 'triplets' were then introduced into the uteri of (SJL × C57BL/10)_F pseudopregnant females. To date, 55 'triplet' embryos have been transferred into six pseudopregnant females and two litters have been delivered.

On 30 January 1977 a litter of five mice was delivered by a female that had received eight 'triple' embryos. Of the four live young, one was a male about half the size of his three female littermates. This male was white with small patches of pigmented hair on the back and head that could only have been derived from the parthenogenetic embryos. Some of the pigmented hair was agouti and some non-agouti indicating that the mouse was chimaeric with respect to hair follicle cells. Both irises had pigmented and non-pigmented areas (Fig. 1). No nipples were evident. At 26 d old the chimaeric male weighed 13 g, and his littermates weighed 16 g each.



Fig. 1 Eye of chimaera mouse. The non-pigmented areas were derived from a normally fertilised egg, the pigmented areas from one or two parthenogenetically activated eggs.

At 36 d old, blood samples were collected from the male and his four littermates and analysed for GPI (Fig. 2). All of the non-chimaeric females showed only the GPI-1A form of glucose phosphate isomerase. The red blood cells of the chimaeric male apparently included cells derived from the parthenogenetic embryos as shown by a GPI-1B band (Fig. 2) that could only

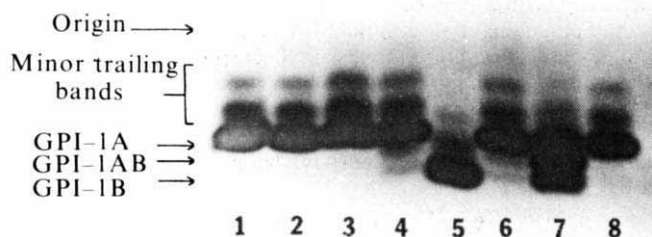


Fig. 2 Electrophoresis of red blood cell lysates on cellulose acetate gels stained for glucose phosphate isomerase using method of Eicher and Washburn¹⁰. In slots 1–3 and 8 are lysates from the four normal female sibs (GPI-1A) of the chimaeric male. Slot 5 includes lysate from a C57BL/6J male (GPI-1B) and slot 7 from a (C57BL/6J × DBA/2J)_F male (GPI-1AB). Slot 4 (repeated in slot 6) contains lysate from the chimaeric male. Of interest are the two bands present in slots 4 and 6 that have migrated at a faster rate than the major band in the position of GPI-1A. These two minor bands line up such that one is in the same position as the GPI-1AB (hybrid) band and the other in the position of the GPI-1B band. It seemed that the slower of the two bands (position of GPI-1AB hybrid band) stained more intensely than the faster band (position of GPI-1B), as is the case in lysates from a *Gpi-1^a/Gpi-1^b* individual, suggesting that at least one parthenogenetically-derived embryo in the chimaeric male mouse was of the *Gpi-1^a/Gpi-1^b* genotype.

result if cells of parthenogenetic origin were present. In addition, a GPI-1AB band was evident. Its intensity seemed greater than the GPI-1B, as is the normal case of *Gpi-1^a/Gpi-1^b* cells, suggesting that at least one of the parthenogenetic embryos was of the *Gpi-1^a/Gpi-1^b* genotype. The presence of a GPI-1AB band would imply that at least one of the parthenogenetic embryos resulted from mitosis of a primary oocyte or lack of a second meiotic division in a secondary oocyte (with crossing over between the centromere and the *Gpi-1* locus), but not diploidisation of haploid ootids. It is also possible that a polar body nucleus fused with its egg nucleus.

At 38 d of age the male gonads were examined through an incision in the abdominal skin and musculature. Both seemed to be normal testes. The right testis was removed and prepared for histological examination. There was no histological evidence that cells of parthenogenetic origin were present in the testis. To date the chimaeric male has sired 27 albino offspring, none of which shows evidence of parthenogenetic cells.

On 27 March 1977, a second litter of three mice was delivered. This litter was obtained by transferring 9 'triple' embryos each composed of two LT/Sv parthenotes with one albino 129 embryo. The female of this litter was much smaller than its two male littermates. Both her irises were pigmented showing the presence of parthenogenetic cells.

The evidence presented here shows that cells of parthenogenetic origin can survive and participate in normal organ formation. Since both parents of the normal embryo were albino, all of the pigment cells in the coat and irises of both chimaeras must have been derived from parthenogenetic embryos. Since the agouti pattern is determined by the genotype of the hair follicle cells, the presence of agouti hairs in the male chimaera indicates that some of these cells originated from the parthenogenetic member. Finally, the presence in the male chimaera of LT/Sv strain-specific GPI shows that red blood cells were derived from parthenogenetic embryos of the chimaera.

Although our preliminary results indicate that parthenogenetic cells are capable of differentiating normally in combination with normal cells, it is still not clear why parthenotes cannot survive to term *in utero* in strain LT/Sv mice.

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Note added in proof: It has recently been found that ovarian teratomas in mice are derived from oocytes that have completed the first meiotic division¹¹.

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Ovarian teratomas in mice are derived from oocytes that have completed the first meiotic division

SPONTANEOUS ovarian teratomas are found in about 50% of strain LT/Sv mice by the time they are 90 d old¹. These teratomas result from parthenogenetic cleavage of ovarian oocytes. Some parthenotes reach a developmental stage equivalent to 7 d embryo before they become disorganised and further develop into a teratoma. Since some cleaved ovarian oocytes are accompanied by polar bodies, but others seem to lack them, it was uncertain whether the teratomas derive from oocytes that complete the first meiotic division or from oocytes that cleave mitotically without previous meiotic division.

A strain of mice having an even higher frequency of ovarian teratomas than LT/Sv was produced by making a recombinant inbred line from strains LT/Sv and C57BL/6J (L.C.S. and D.S. Varnum, unpublished). Nearly all of the females of this recombinant line, called LTXBJ, have bilateral ovarian teratomas by 90 d of age. Fortuitously, among the C57BL/6J genes retained in the recombinant strain is the *Gpi-1^b* allele at the glucosylphosphate isomerase (*Gpi-1*) locus on chromosome 7. Since strain LT/Sv is homozygous for the *Gpi-1^a* allele at this locus, (LT/Sv × LTXBJ)_{F1} hybrids are heterozygous at *Gpi-1* and express the A, hybrid AB and B allozymes in a ratio of 1:2:1 (Fig. 1a).

Electrophoresis of 23 teratomas from *F1* female mice revealed a homozygous A or B allozyme banding pattern in 21 cases (Fig. 1b). The other two teratomas showed the 1:2:1 heterozygous banding pattern, indicating that they were heterozygous *Gpi-1^a/Gpi-1^b*.

We concluded from these results that the teratomas found in the (LT/Sv × LTXBJ)_{F1} female mice originated from parthenogenetically cleaved oocytes which had completed the first meiotic division (Fig. 2). In 21 of 23 cases, this division segregated the *Gpi-1^a* and *Gpi-1^b* alleles so that the parthenogenetic embryo and subsequently the teratomas were homozygous for either of those alleles at the *Gpi-1* locus. In the other two cases, where the heterozygous banding pattern was found, the most probable conclusion is that crossing-over has occurred between the centromere and the *Gpi-1* locus, so that the *Gpi-1^a* and *Gpi-1^b* alleles

were located on two chromatids attached to the same centromere.

Other studies on the origin of ovarian teratomas in women who were heterozygous for alleles producing allozymes of glucose-6-phosphate dehydrogenase and phosphoglucomutase, have shown that these teratomas were homozygous for the alleles in most cases, and thus that they arose from oocytes that had completed the first division of meiosis².

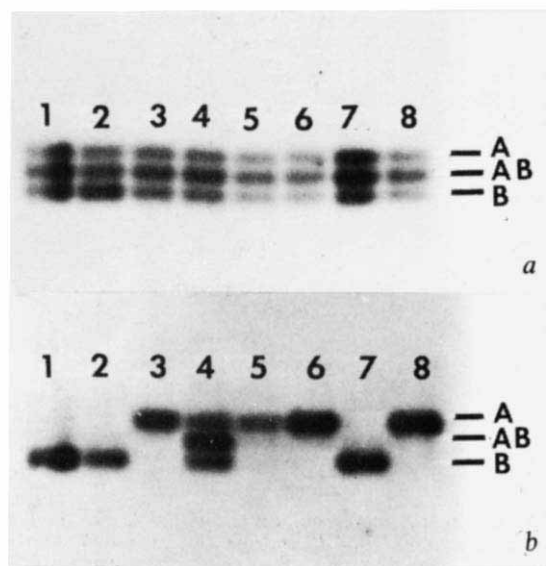


Fig. 1 a, Electrophoresis of supernatants prepared from ovaries (slots 1-6, and 8) and spleen (slot 7) from (LT/Sv × LTXBJ)_{F1} mice. Male LTXBJ mice were mated with female LT/Sv to produce the hybrids. At 8-10 weeks of age, the *F1* females were autopsied and teratomas were removed while taking care to contaminate the tumour with as little ovarian tissue as possible. Excessively bloody tumours were not used. Pieces of tissue were homogenised with ground glass microhomogenisers in 0.05 ml 50 mM Tris-HCl buffer, pH 7.5, containing 1 mM EDTA and 1 mM β-mercaptoethanol. The homogenate was centrifuged for 20 min at 16,000g and the supernatant solution used as the enzyme source. Electrophoresis was carried out on Titan-III Zip Zone cellulose acetate plates (Helena Laboratories, Beaumont, Texas) with 0.043 M Tris, 0.046 M glycine buffer, pH 8.6, for 1.75 h at 180 V. Glucose phosphate isomerase (EC 5.3.1.9.) was detected with a 1% agar overlay containing 50 mM Tris-HCl, pH 8.0, 2.5 mM MgCl₂, 2.5 mM fructose-6-phosphate, 1 mM NADP, 0.1 mg ml⁻¹ phenazine methosulphate, 0.5 mg ml⁻¹ nitroblue tetrazolium and 5 IU ml⁻¹ glucose-6-phosphate dehydrogenase. The GPI-1B form in slot 2 appears darker than the GPI-1A, suggesting that this piece of ovary was contaminated with some teratoma tissue which was not obvious when the sample was taken. A piece of *F1* tissue was tested from each animal from which a teratoma was assayed to assure heterozygosity. b, Electrophoresis of supernatants prepared from teratomas from (LT/Sv × LTXBJ)_{F1} female mice. Samples 1-3 and 5-8 show either the GPI-1A or GPI-1B allozyme pattern. Sample 4 is one of the two teratomas that showed a hybrid allozyme pattern. Samples 7 and 8 were taken from contralateral ovaries of the same animal (slot 7, Fig. 1a) and are homozygous for alternative allozymes. In some teratomas, the hybrid AB allozyme and the other homozygous allelic product were detected, but in these cases the amount of the AB form greatly exceeded the minor homozygous form, indicating that the tumour was probably contaminated with normal ovarian tissue.

Preliminary chromosomal analysis of LT teratoma has indicated that most of the cells are diploid and a small number are polyploid (L.C.S. and D.S. Varnum, unpublished). Also, the parthenotes recovered from the oviduct of superovulated LT/Sv mice are also diploid, although at least one polar body was usually found (P. C. Hoppe, personal communication). Therefore, diploidisation of the egg karyotype must have occurred after the first division. The mechanism of this diploidisation is unknown but it is possible, in principle, that karyokinesis of the second

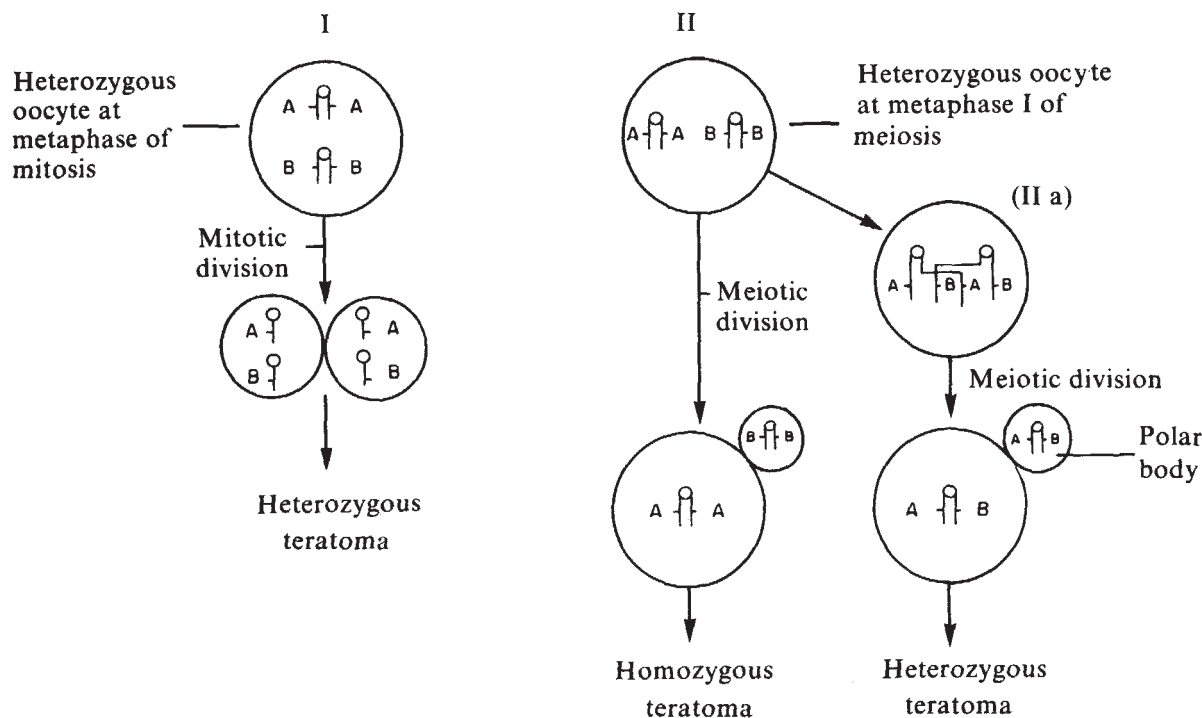


Fig. 2 Diagrammatic representation of alternatives for the distribution of alleles at the *Gpi-1* locus (A and B refer to the *Gpi-1^a* and *Gpi-1^b* alleles, respectively). The GPI enzyme consists of two subunits which associate randomly. Consequently, homozygous *Gpi-1^a* or *Gpi-1^b* cells contain enzyme with two A or B subunits, respectively. Heterozygous *Gpi-1^{ab}* cells contain three forms of the enzyme: one with two A subunits, one with an A and a B subunit, and one with two B subunits in a 1 : 2 : 1 ratio. If teratomas originate from oocytes which cleave mitotically without previous meiotic division (alternative I), then all teratomas would show the heterozygous banding pattern for GPI. If, however, the first meiotic division occurs before parthenogenetic cleavage, then (as the data presented here indicates) most teratomas would be homozygous for either the GPI-1A or GPI-1B allozymes (alternative II). In a minority of cases, heterozygous teratomas would be found resulting from a chromatid exchange between the *Gpi-1* locus and the centromere (IIA).

meiotic division occurs without cytokinesis, thus suppressing the formation of polar body II; or that there is a fusion of the second polar body with the ovum. Our preliminary results do not allow us to distinguish between these possible alternatives.

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Extreme sensitivity of some intestinal crypt cells to X and γ irradiation

THE destructive effects of radiation have been studied for 80 yr. Most techniques involve looking at the surviving cells, which tend to be the more resistant cells of the tissue. On the assumption that the results are representative of all cells in the tissue, many conclusions have been drawn. On the other hand, Cheng and Leblond have used tritiated thymidine ($^3\text{HTdR}$) to kill cells synthesising DNA in the crypts of the small intestine¹. Two surprising features of their experiments have provoked little comment. First, very low doses (40-50 μCi per mouse) of $^3\text{HTdR}$

caused measurable cell killing and second, the killing (evident from the presence of labelled apoptotic-like² phagosomes¹) was not random throughout the crypt but occurred selectively at the crypt base where relatively few cells are in S (refs 3 and 4) and where the stem cells are presumably located^{1,3-5}. I report here that the presence of hypersensitive cells at the base of the crypts can be demonstrated after whole-body X or γ irradiation, and to describe the time sequence for the production and loss of these killed cells together with their dose-response relationship.

Figure 1 compares the changes in the number of apoptotic cells in sections of the whole crypt and in sections of the lower third of the crypt after whole-body irradiation. Peak values are obtained within 3-6 h, with 2-3 of the total apoptotic bodies occurring in the lower third. Because the upper third contains few apoptotic bodies after 3 or 6 h this represents a twofold difference in the yield in the base and mid-crypt region. The difference in sensitivity may be much greater for certain cell positions because the base region contains the mature Paneth cells which do not contribute extensively to the apoptotic yield. The control level of apoptoses fluctuates slightly with the time of day but is not affected by handling of the animals (saline injections) or sham irradiation (Fig. 1).

Apoptotic bodies in the crypt base can be ingested and gradually absorbed by neighbouring Paneth cells, stem cells or amplifying committed cells^{1,3-5}. In the last case they will be carried rapidly into the mid-crypt region and then out of the crypt on to the villus. For higher doses the apoptotic yield may remain at elevated levels for longer times because cell cycle progression, crypt transit and cell movement may be retarded when many cells are killed; furthermore, other more radiation-resistant cells may take longer to appear apoptotic. In the case of ingestion by Paneth or stem cells, the apoptotic bodies will remain in the basal region for a longer time, either until absorbed to a residual body no longer detectable in the light microscope (Paneth cells) or until passed by stem cell division into the cytoplasm of a new committed cell

(because stem cells cycle more slowly than the committed cells^{1,3-7}). For these reasons sampling was restricted to either 3 or 6 h after irradiation and in many cases the average of both 3 and 6-h samples was used.

Figure 2 shows the dose dependence for the yield of apoptotic cells in the lower third of the crypt 3 h after irradiation. There was a fivefold increase in yield after 1–2 rad and a tenfold increase after 5 rad. Beyond 20 rad the yield increased more slowly. Because the points are somewhat scattered it is not clear whether the yield was constant beyond 20 rad or continued to increase gradually. The highest values were obtained after a dose of 900 rad and represent a 30–40-fold increase. Beyond 900 rad, crypt survival seems to be related exponentially to dose⁸⁻¹⁰, with a D_0 value of 100–200 rad⁸⁻¹⁰. Thus a few cells (perhaps one to three) survived with an intact reproductive potential after 900 rad, that is 98–99% of the 250 crypt cells were sterilised. But only a very small fraction of these sterilised cells appeared apoptotic.

Figure 3 shows the same data in greater detail with the crypt base and total crypt apoptotic yields using the average of both the 3- and 6-h samples. The points show less scatter and illustrate that

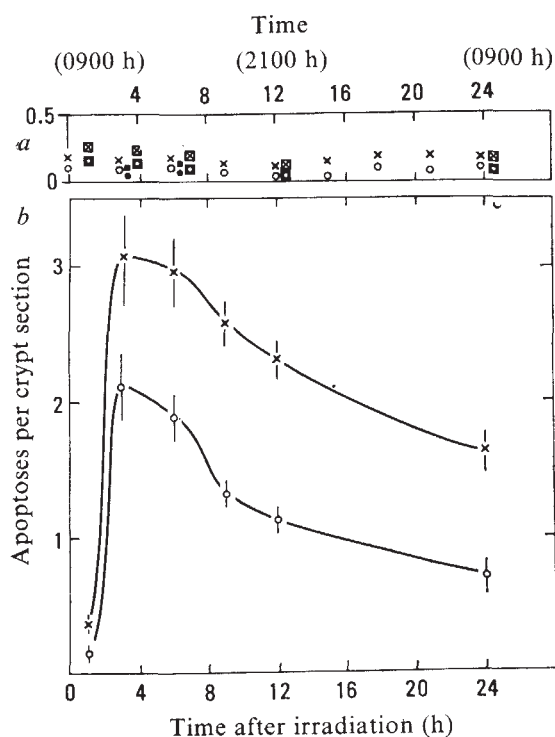


Fig. 1 Apoptotic yield in unirradiated mice (a) and after 63 rad whole-body ^{137}Cs γ irradiation (520 rad min^{-1}) (b). Each point shows the mean and standard error of at least four individual mouse values. Scoring was done at $\times 500$ magnification on haematoxylin and eosin 5–7 μm paraffin sections. Forty crypt sections were scored from each mouse. Crypts were scored only if they were well sectioned longitudinally with the Paneth region, a clear lumen and at least 17 cells up one side of the crypt (the mean number of cells up one side was about 22). The crypts were then divided into three regions, each of approximately 1/3 the total number of cells. It is assumed that the number of apoptotic cells was directly proportional to the number of cells killed over the range of radiation doses studied. Because apoptotic cells tend to be centripetally positioned and disperse slightly a good longitudinal crypt section yields about 60% of the apoptoses per crypt (as revealed by comparing sections and whole squashed crypts). Thus the total number of hypersensitive cells per crypt would have been about 4.5 (based on the value at 40 rad). Groups of untreated animals were killed at various times of the day ($\times$, total; $\circ$, base), at various times after an intraperitoneal injection of physiological saline (0.2 ml) (cross in square, total; circle in square, base), and 3 and 6 h after sham irradiation ($\blacksquare$, total; $\bullet$, base). In none of these cases did the total apoptotic yield exceed 0.25 per crypt section (overall mean 0.17 total and 0.08 crypt base). There was thus an approximately 20-fold increase in total apoptoses within 3 h of irradiation. The proportion of apoptotic cells is even larger when the crypt base is considered.

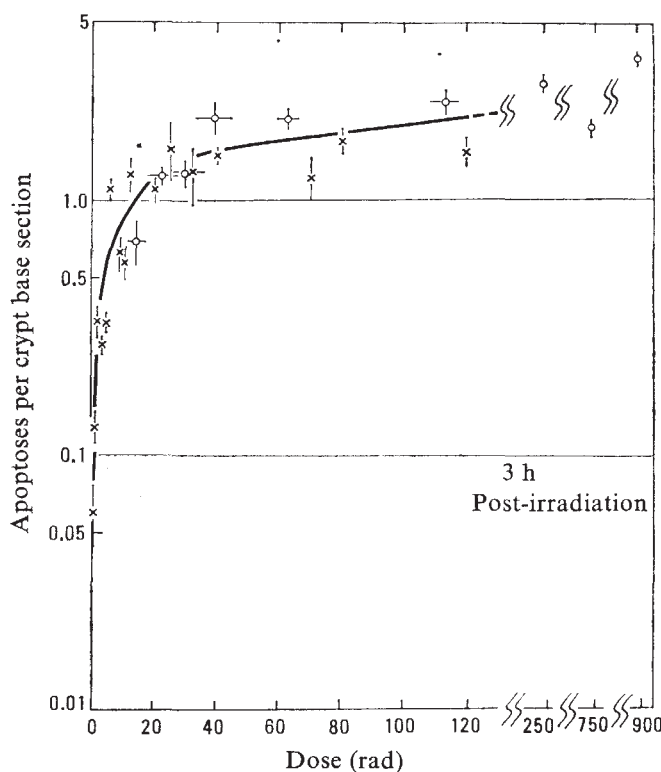


Fig. 2 Dose-response for apoptotic cells in the lower third of the crypt 3 h after irradiation. Apoptotic yield is plotted on a logarithmic scale against dose on a linear scale. Each point shows the mean, with its standard error, of at least four mice. $\circ$, Values for ^{137}Cs γ rays (520 rad min^{-1}); $\times$, 290 or 300 kVp X rays (30 rad min^{-1}). The errors on the doses were estimated to be about $\pm 5\%$ for all doses except the lowest γ -ray doses where it was not more than $\pm 20\%$.

radiation hypersensitivity was confined largely to cells in the base of the crypt. These results clearly illustrate the presence of some extremely sensitive cells in the lowest region of the crypt—in the region immediately above the Paneth cells and below most of the rapidly proliferating cells, that is, within the presumptive stem cell region^{1,5-7}. The number of hypersensitive cells per control crypt section is uncertain. If it is assumed that most of the hypersensitive cells are killed by 40 rad, which is just on the resistant portion of the dose-response curve shown in Fig. 3, the number of apoptoses observed after 40 rad would be close to the total number of hypersensitive cells present in a crypt section. The data (from Fig. 3) can thus be expressed as survivors rather than killed cells and a survival curve can be generated (Fig. 4). The resulting points are scattered about a line with a 50% effective dose of 8 rad and a D_0 of about 10 rad. This places these crypt cells among the most sensitive mammalian cells. Figure 4 shows that the most sensitive type of oocytes (stage 1) have a similar sensitivity¹² while the most sensitive spermatogonia (preleptotene)¹³ and the sensitive cortical thymic lymphocytes¹⁴ are more resistant than the crypt cells. An extreme sensitivity can also be seen in some early mouse embryo cells after doses in range 5–25 R (refs 15–17). This survival curve is speculative and the actual slope (D_0) depends somewhat on the value used for the number of hypersensitive cells per crypt, but whatever way the data are expressed, within the stem cell region at the crypt base there are clearly a few extremely radiosensitive cells. Preliminary observations indicate the presence of similar hypersensitive cells in the growing hair follicle matrix and colonic crypts.

The precise function of the radiosensitive cells in the crypt is uncertain. They do not represent any known histologically distinctive cell type (at the level of either light or electron microscopy). Their presence suggests at least two radiobiologically distinct crypt cell populations. If they are stem cells they must represent a subpopulation of the total complement of potential

stem cells (about 80 per crypt⁹) because the crypts are not destroyed when these hypersensitive cells are killed. The number of functional stem cells per crypt is uncertain but is probably about 20, that is about six times the number of hypersensitive cells. The subpopulation of hypersensitive cells might be at some extremely sensitive stage of the cell cycle peculiar to stem cells. The normal sensitivity changes through the cell cycle are relatively small and the sensitive phases (G_2 and M)¹⁸ do not exhibit sensitivities such as those shown here. It is not clear whether these apoptotic bodies are formed from interphase cells or from abortive divisions. In the latter case their appearance within 3 h could suggest a hypersensitive point somewhere in G_2 .

One rad of radiation of this quality produces sufficient ionising tracks for at least one track to cross some part of about 50% of the cell nuclei (assuming an average diameter of about $4\mu\text{m}$). The average track length per rad per cell nucleus is about $2\mu\text{m}$. The average energy deposition per nucleus is 2KeV rad^{-1} . The data in Fig. 3 suggest that after 1 rad one sensitive cell in ten will have at least one track traversing its critical volume which might be about $1.4\mu\text{m}$ in diameter (that is about 1/20 of the nuclear volume) and that one track crossing this volume is sufficient to kill that cell.

The mechanism by which these sensitive cells are killed is unknown, but DNA damage can be detected at similar doses (up to 5 rad)¹⁹. It has been suggested that stem cells would benefit from a mechanism by which newly synthesised DNA strands (containing replication errors) were selectively distributed to the committed cells and thus eliminated, ensuring the genetic stability of the stem cell DNA²⁰. In these conditions damage to the stem cell template DNA might be intolerable and the cells might die. The extreme sensitivity seen here would suggest that any DNA damage would result in death; furthermore that other cells differing in the way they normally handle their DNA may assume the role of regenerative stem cells which would be a potentially hazardous process because possibly imperfect DNA would be 'immortalised'. This assumes that the vital stem cells lack any means of repairing genetic errors. An alternative explanation implies the

Fig. 3 Dose-response for apoptotic cells in the lower third (base, $\times$ and $\circ$) and the total crypt ($\bullet$). The individual data points are the mean of 3- and 6-h post-irradiation groups for X rays ($\times$) and γ rays ($\circ$). The insert shows the first nine data points for the base (0–12 rad).

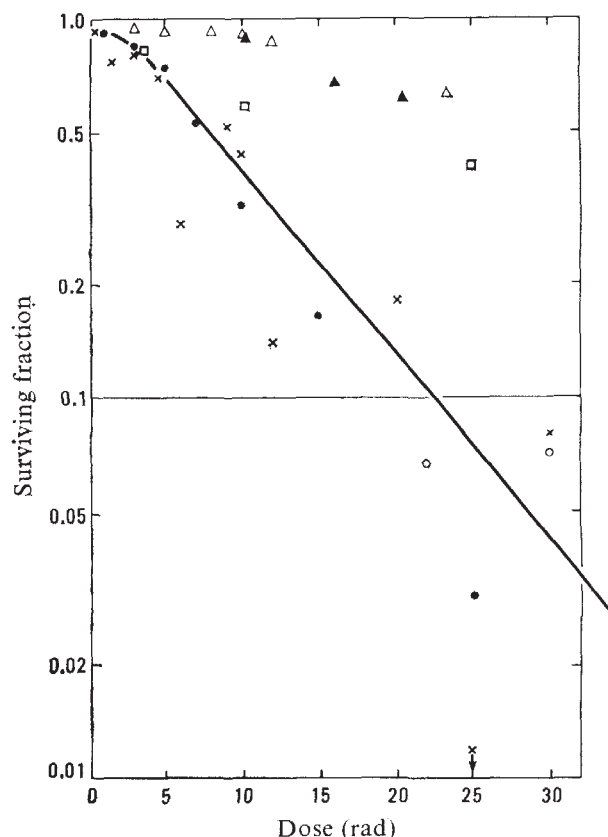
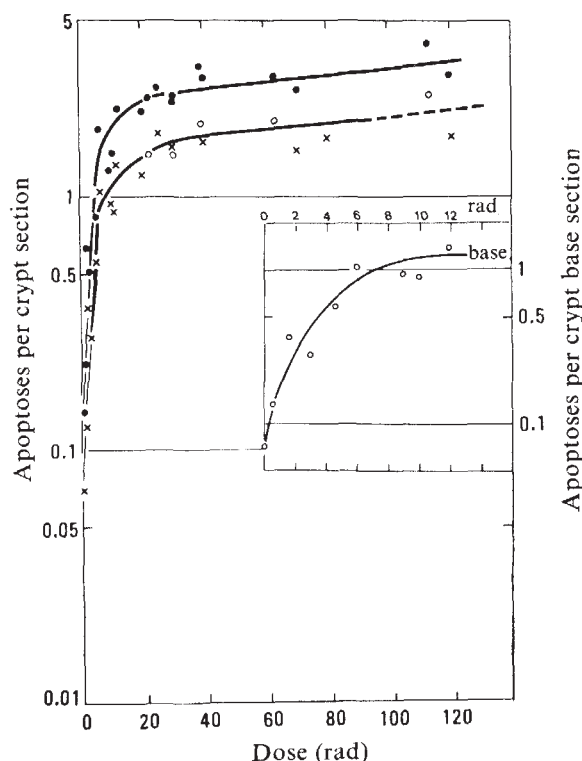


Fig. 4 Survival curve for apoptotic sensitive crypt cells ($\times$, X rays, $\circ$, γ rays). Surviving fraction is plotted on a logarithmic scale against radiation dose on a linear scale. The points were obtained by assuming that there was an average of 2.7 apoptotic sensitive cells per crypt section (value reached at 40 rad, see Fig. 3). Data points for stage 1 oocytes¹² ($\bullet$), preleptotene spermatogonia¹³ (Δ), type B spermatogonia ($\blacktriangle$) and cortical thymic lymphocytes¹⁴ ($\square$) have also been plotted. The line has been fitted by eye and has a D_{01} of approximately 10 rad.

reverse, that the stem cells possess an extremely efficient means of restoring the DNA integrity; the stem cells may possess a mechanism for detecting template errors and (1) transferring G_1 induced errors by means of sister chromatid exchanges and mitosis to the committed daughter which subsequently dies, or (2) eliminating G_2 -induced errors by elevating the newer strands to stem cell template status and discarding the damaged DNA to a cell that subsequently dies. Any of these possibilities could explain the apparent indifference of the crypt as a whole to this type of death.

The significance of this phenomenon in public health (maximum permissible doses)—doses within the range studied here are delivered in several diagnostic radiological procedures—and radiotherapy, depends on the function of the hypersensitive cells and the mechanism by which they are killed.

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Cellular serine proteinase induces chemotaxis by complement activation

INFILTRATION of inflamed or injured tissues by polymorphonuclear leukocytes is a fundamental pathophysiological response. Undoubtedly, there are numerous mechanisms by which leukocytes are attracted to an area of damage. One possibility is that cellular injury could release or activate a proteolytic enzyme which could generate chemotactic factors. Extracts of parenchymatous tissues¹ and cells² are chemotactic when they are incubated with complement sufficient serum. Macrophages³ and leukocytes⁴ contain a proteinase which generates chemotactic peptides, and recently human polymorphonuclear leukocytes have been shown to secrete an enzyme which activates complement⁵. Lazarus and Barrett extracted and characterised a serine proteinase which induced polymorphonuclear leukocyte infiltration of the skin when injected intradermally⁶. Subsequently, this proteinase, which is both cytotoxic and phlogistic, has been purified to homogeneity from whole human skin⁷, human epidermis⁸, human lymphocytes⁹ and cultures of newborn mouse epithelium (G.S.L., unpublished). This report demonstrates that this proteinase is significantly more active than trypsin, plasmin or Pronase in generating polymorphonuclear leukocyte accumulation and that chemotactic activity is largely dependent on complement activation.

The procedure for purification of the enzyme has been described previously⁷; this was used with several modifications. Frozen human skin, obtained from amputated limbs, was minced and added to 10 volumes 50 mM phosphate buffer, pH 7.4, containing 1.0 M potassium chloride. The mixture was frozen and thawed five times and further extracted by agitation at 4 °C for 18 h. After centrifugation at 50,000g, the supernatant was subjected to 85% saturation with ammonium sulphate and the proteinase was extracted from the precipitate with a minimum volume of extraction buffer. The extract was concentrated and placed on a Sephadex G-75 superfine column and the eluted fractions containing the smallest molecular weight proteinase (molecular weight approximately 28,000) were combined and further purified by chromatography on a soybean trypsin inhibitor–Sephadex affinity column.

This purification method yielded a single protein band on polyacrylamide gel electrophoresis and SDS–polyacrylamide gel electrophoresis. Injection of the 250-fold purified enzyme into rabbits produced an antibody which gives a single line of identity in Ouchterlony double-diffusion plates against purified enzyme

and crude starting material. The enzyme was inhibited by diisopropyl fluorophosphate (DFP), soybean trypsin inhibitor, α_2 -macroglobulin and α_1 -antitrypsin. Further characterisation has demonstrated that it was not elastase, cathepsin G or plasminogen activator.

Polymorphonuclear leukocyte chemotaxis was assayed by the method of Snyderman *et al.*¹⁰. Sample (2 ml) was injected into the peritoneal cavity of a group of normal or C5-deficient male mice (Jackson Laboratories, Bar Harbor, Maine). After 12 h, the animals were killed by inhalation of carbon dioxide, and the peritoneal cavity was vigorously washed with 9 ml Dulbecco's Modified Eagle's Media with 10% foetal calf serum (GIBCO), containing 90 IU herapin (Upjohn). The peritoneal wash was counted for total number of white cells and for percentage polymorphonuclear leukocytes (PMN) by standard techniques. Results were expressed as absolute number of PMN. All assays were carried out in triplicate on three occasions, and the materials tested included saline controls, proteose peptone, a complement-independent chemotactic agent (9%, DIFCO Laboratories), and proteinase with and without inhibitors. Proteolytic activity of all materials was assayed by measuring production of trichloroacetic acid-soluble peptides from tritium-labelled casein⁸.

Polymorphonuclear leukocyte accumulation was directly proportional to enzyme concentration in the range 1–50 μ g enzyme protein. Table 1 compares the ability of the purified human proteinase to elicit polymorphonuclear leukocyte accumulation with other common proteolytic enzymes operative at neutral pH. Our enzyme (20 μ g) induced a brisk accumulation of polymorphonuclear leukocytes. Pre-incubation of enzyme with diisopropyl fluorophosphate (1 mM final concentration, 1 h, 25 °C) followed by exhaustive dialysis against physiological saline, almost completely inhibited polymorphonuclear leukocyte accumulation. These data suggest that enzymatic activity is necessary for polymorphonuclear leukocyte accumulation. By contrast, injection of 200 μ g plasmin, Pronase or trypsin attracted one-third the number of polymorphonuclear leukocytes into the peritoneum. This was not simply a function of increased proteolysis by our enzyme; expression of polymorphonuclear accumulation per amount of casein-degrading activity, revealed that our purified skin proteinase was 3–5 logs more effective in attracting polymorphonuclear leukocytes on a proteolytic activity basis than the other proteinases tested.

We next attempted to elucidate the mechanism by which our purified human proteinase induced such a significant polymorphonuclear leukocyte accumulation. Equal amounts of the purified proteinase were injected into normal and C5-deficient mice (Fig. 1). The enzyme evoked the expected dramatic response in normal mice, which was almost completely inhibited by DFP. By contrast, the enzyme was a quarter as effective in evoking polymorphonuclear leukocyte infiltration in C5-deficient mice. Furthermore, the minimal chemotactic response in C5-deficient mice could be reduced to control levels by pre-incubation of the enzyme with DFP. These data show that the enzyme evokes its chemotactic response principally through the complement cascade and probably requires the production of C5 fragmentation for maximal activity. The data also suggest that the proteinase is capable of inducing limited chemotaxis by a C5-independent mechanism.

Table 1 Relative chemotactic activity of proteolytic enzymes

	μ g Injected	Proteolytic activity (c.p.m. TCA-soluble protein)	PMN per mouse $\times 10^{-4}$	Chemotactic index PMN/c.p.m. proteolytic activity
Human skin proteinase	20	1.2×10^3	370	3,060
Plasmin	200	3.5×10^5	90	2.6
Pronase	200	2.8×10^7	126	0.05
Trypsin	200	6.2×10^7	128	0.02

Proteinases were assayed for proteolytic activity before injection and polymorphonuclear leukocyte (PMN) accumulation was measured 12 h after injection. Chemotactic index expresses PMN accumulation per unit of proteolytic activity.

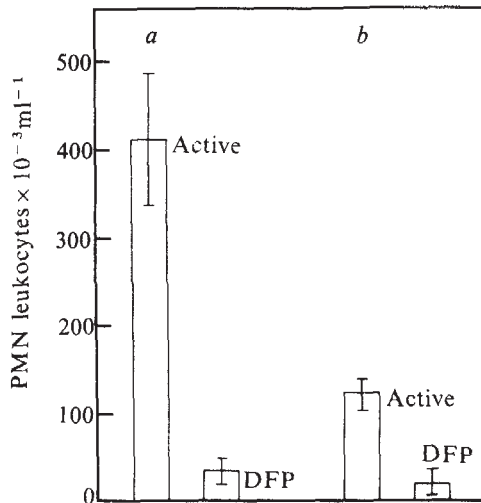


Fig. 1 Purified human proteins (20 μ g) and a comparable amount of enzyme inhibited with DFP, were injected into normal (a) and C5-deficient mice (b). Polymorphonuclear (PMN) leukocyte accumulation was determined 12 h after injection.

Our data suggest that cells contain a proteinase operative at neutral pH which is extraordinarily effective in activating complement and inducing polymorphonuclear leukocyte accumulation. The presence of this enzyme in whole skin⁷, human epidermis⁸, human lymphocytes⁹ and mouse epithelial cells in culture (G.S.L., unpublished), could suggest that cellular injury might activate this proteinase, which through the mediation of complement induces polymorphonuclear leukocyte accumulation. Such a mechanism might explain how cellular injury induces polymorphonuclear leukocyte accumulation.

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The ECF-A tetrapeptides and histamine selectively enhance human eosinophil complement receptors

VARIOUS products of the anaphylactic reaction such as the ECF-A tetrapeptides¹ (Val-Gly-Ser-Glu and Ala-Gly-Ser-Glu)² and histamine^{3,4} preferentially attract the human eosinophil in chemotaxis *in vitro*. We have designed experiments to show whether these chemical mediators have a direct effect on the eosinophil cell membrane, as assessed by their capacity to alter receptors for either IgG or complement (C). We have established here that these two ECF-A peptides and histamine both enhance markedly the expression of eosinophil receptors for C3 but not for IgG. Neutrophil and monocyte receptors seemed to be unaltered and a number of other chemical mediators of hypersensitivity had no effect on these membrane markers when tested at comparable concentrations. Our demonstration of eosinophil membrane receptor enhancement by chemotactic factors may have more general biological significance in terms of how surface recognition mechanisms by phagocytic cells are regulated.

Receptors were measured by the 'rosette technique' using sheep red blood cells (RBC) sensitised with either IgG or C3b (Fig. 1). Human eosinophils, neutrophils⁵ and monocytes⁶ were prepared from venous blood from patients with eosinophilia of various aetiology and adjusted to a concentration of 4×10^6 ml⁻¹ in

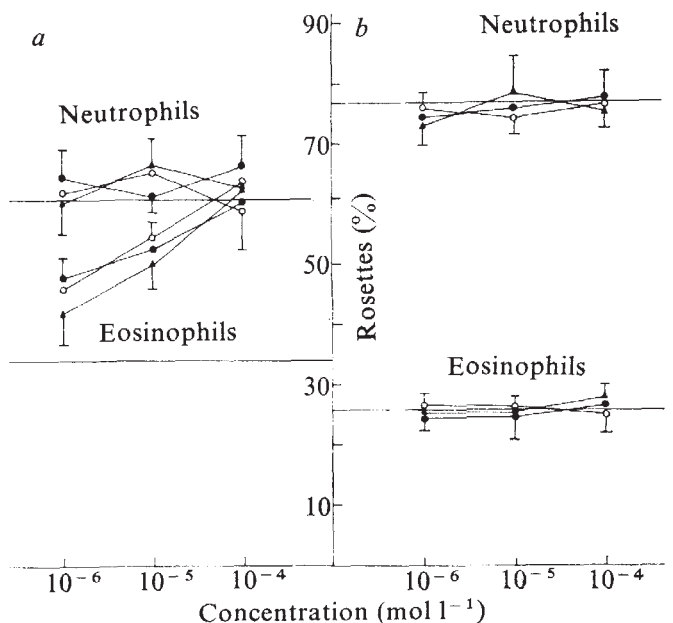


Fig. 1 The effect of increasing concentrations of the valyl-(●) and alanyl-(○) ECF-A peptides and histamine (▲) on a, EAC3b and b, EAG rosettes by human eosinophils and neutrophils. The lines represent the percentage rosettes of untreated eosinophils and neutrophils. Each point represents the mean of five experiments $\pm$ 1 s.d. Dextrose gelatin-veronal buffer (DGVB²⁺ pH 7.4) was used for washing sheep RBC during sensitisation and coating with various complement components. This was prepared by mixing equal volumes of isotonic veronal-buffered saline (containing 0.0015 M Ca²⁺, 0.0005 M Mg²⁺ and 0.1% gelatin-veronal buffer (GVB²⁺) with 5% dextrose in water containing the same concentration of Ca²⁺ and Mg²⁺ (ref. 10). The IgG and IgM fractions of rabbit antisera to sheep red cells were prepared by Sephadex G-200 gel filtration. Sheep cells were sensitised with either the IgG fraction for preparing EAC_G^{rab} (EAG) or IgM (EAM^{rab}) for EAC1423b rosettes. Functionally pure human complement components were added sequentially to EAM^{rab} to prepare C3b-coated cells as described¹¹. The amounts were as follows: 400 effective molecules of C1, 400 of C4, 50 of C2 and 2,500 of C3. This amount of C4 was insufficient to give EAC14 rosettes with neutrophils, eosinophils or monocytes¹¹. The valyl- (HCl)-Val-Gly-Ser-Glu, molecular weight 427) and alanylpeptides ((HCl)-Ala-Gly-Ser-Glu, molecular weight 497) were a gift from Dr R. Camble (ICI Limited, Pharmaceuticals Division, Alderley Park, Macclesfield; histamine acid phosphate (BDH Chemicals Limited, Poole).

medium 199 (pH 7.4). Equal volumes of leukocyte suspensions and various concentrations of the pharmacological agents under study, or control medium alone, were mixed and incubated in a shaking water-bath at 37 °C for varying intervals. The cells were then washed twice in medium 199 and the numbers adjusted to $2 \times 10^6 \text{ ml}^{-1}$ in the same medium. An aliquot (0.1 ml) of IgG-(EAG) or complement-coated (EAC3b) red cells containing $1 \times 10^8 \text{ ml}^{-1}$ was then added to 0.1 ml of the cell suspension. The mixtures were centrifuged at 100g for 10 min at 4 °C and the pellets incubated at 0 °C (for EAG rosettes) or 37 °C (for EAC3b rosettes) for 30 min as described. The pellets were gently resuspended and smears were prepared in duplicate. The slides were dried in air, fixed in methanol and stained. Cells with three or more adherent erythrocytes were counted as rosettes. Two hundred cells were counted on each slide and the number of rosettes expressed as a percentage of the total number counted.

With no treatment the percentages of neutrophil, IgG or complement receptors were usually more than twice that of the eosinophil (Fig. 1). With increasing concentrations of the valyl- or alanyl-peptide, or histamine there was a dose-dependent enhancement of the numbers of eosinophils, but not of neutrophils, which formed rosettes with EAC3b. There was no increase in the numbers of eosinophils or neutrophils forming rosettes with EAG. When other pharmacological mediators, which included bradykinin and the prostaglandins PGE_1 , E_2 and $\text{F}_{2\alpha}$, were incubated at comparable concentrations (10^{-4} – $10^{-6} \text{ mol l}^{-1}$) to that of the ECF-A peptides or histamine, there was no appreciable increase in the numbers of eosinophil- or neutrophil-EAC3b rosettes (Table 1). An increase of 25% was observed with 5-hydroxytryptamine, but only at the highest concentration ($10^{-4} \text{ mol l}^{-1}$). The enhancement with the ECF-A peptides or histamine, with this dose was almost three times this value, thus achieving virtually the same percentage of rosetting eosinophils as neutrophils (Figs 1 and 2). The monocyte was also tested with the valyl- and alanyl-peptide and histamine but, unlike the eosinophil, there was no apparent alteration in the numbers of rosetting cells (Table 1). The enhancement of EAC3b eosinophil rosettes by the ECF-A peptides or histamine also increased with incubation time (Fig. 2). With the peptides, however, most of the increase in rosette formation was apparent at 40 min. In contrast, most of the histamine-induced rosette enhancement took place between 40 and 80 min.

These experiments suggest that the ECF-A peptides and histamine not only attract eosinophils selectively in chemotaxis but also have a direct effect on the eosinophil membrane as shown by the apparent increase in the numbers of complement receptors. It is not known whether this enhancement is caused by the generation of new receptors or whether receptors, previously 'hidden', are revealed as a result of membrane changes. The inability of these pharmacological agents to increase neutrophil and monocyte complement receptors emphasises further the unique, intimate relationship between the human eosinophil and

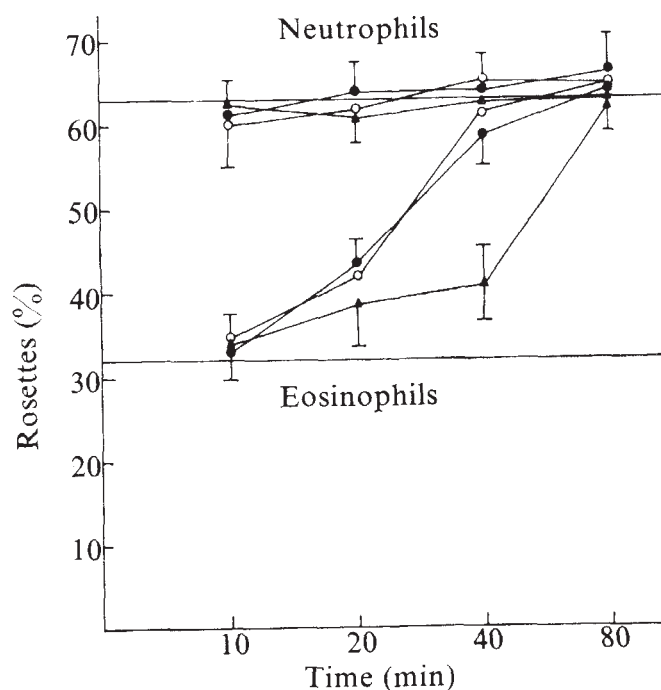


Fig. 2 The effect of time on enhancement of EAC3b rosettes by eosinophils and neutrophils following incubation with the valyl-(●) and alanyl-(○) ECF-A peptides and histamine (▲). Each mediator was tested at a concentration of $10^{-4} \text{ mol l}^{-1}$. The lines represent the percentage rosettes of untreated eosinophils and neutrophils. Each point represents the mean of five experiments ± 1 s.d.

these anaphylaxis-associated agents. We also explored the possibility that neutrophil and monocyte complement receptor enhancement may be revealed when the amount of C3 on the indicator cell was limited. In experiments in which the quantity of C3 used was adjusted to give approximately half the number of rosetting cells (that is, 1,000 effective molecules) however, there was no apparent increase in rosette formation following incubation with these agents. Similar negative results were found with neutrophils and monocytes when the amounts of IgG were decreased to give lower numbers of EAG rosettes. The reason why complement, and not IgG, receptors were altered by the ECF-A peptides and histamine is unknown. It was previously shown that IgG and C3b receptors act together to aid macrophage phagocytosis⁷. A similar mechanism may exist for eosinophils; optimal receptor ratio being determined by the presence of pharmacological mediators such as ECF-A and histamine.

The role of the eosinophil in immediate-type hypersensitivity is thought to be that of a homeostatic cell affecting mediator release.

Table 1 Percentage increase in the numbers of rosetting eosinophils, neutrophils and monocytes with EAC3b cells following incubation with increasing concentrations of various chemical mediators of hypersensitivity

	$10^{-6} (\text{mol l}^{-1})$			$10^{-5} (\text{mol l}^{-1})$			$10^{-4} (\text{mol l}^{-1})$		
	Eosinophils	Neutrophils	Monocytes	Eosinophils	Neutrophils	Monocytes	Eosinophils	Neutrophils	Monocytes
Valyl-peptide	43.3	5.6	(6.8)	55.6	0.5	(0.3)	77.8	8.1	(4.5)
Alanyl-peptide	37.0	1.0	5.3	60.6	8.1	(1.8)	87.8	(3.1)	(7.0)
Histamine	25.1	(1.1)	(3.3)	48.8	7.8	(3.3)	83.4	1.3	3.0
Bradykinin	0.5	7.8	—	0.5	(4.9)	—	3.0	2.3	—
PGE_1	5.6	4.2	—	(2.0)	(8.5)	—	14.7	1.3	—
PGE_2	(0.9)	(3.9)	—	6.5	2.9	—	6.8	(7.7)	—
$\text{PGF}_{2\alpha}$	5.6	(3.1)	—	13.3	1.3	—	(3.3)	2.1	—
5-HT	3.0	(5.2)	—	17.8	(3.4)	—	25.0	6.7	—

The figures represent the mean of five experiments, with the exception of bradykinin (three experiments). Decreases in rosette formation are shown in parentheses. Prostaglandins E_1 , E_2 and $\text{F}_{2\alpha}$ were supplied by Dr John Pike, Upjohn Company, Kalamazoo, USA; bradykinin triacetate and 5-hydroxytryptamine were obtained from Sigma, Kingston-upon-Thames, UK.

mediator inactivation and mediator replenishment by mast cells⁸. Their function in helminth disease may be that of a cytotoxic cell requiring the participation of IgG, in an analogous fashion to lymphocyte-antibody-dependent cell-mediated cytotoxicity⁹. Although complement does not seem to be required in this system, it is nevertheless possible that adherence by way of the C3 receptor may amplify the parasitocidal properties of eosinophils. Our observations may therefore, be of relevance in helminth disease where the IgE-mediated release of chemical mediators is well recognised. In any event, our finding of a new biological activity for both the ECF-A peptides and histamine may provide further insight into both the essential biochemical differences between human eosinophils and other blood leukocytes, and the way in which chemical mediators modulate recognition mechanisms by cell membranes.

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Molecular basis for acquired haemoglobin H disease

HAEMOGLOBIN H (Hb H; β_1) disease results from a reduced rate of synthesis of the α chains of human adult haemoglobin (Hb A; $\alpha_2\beta_2$). In the absence of sufficient α chains, excess β chains form β_4 tetramers which are unstable, precipitate and cause a shortened red cell survival¹. Hb H disease is one of the α -thalassaemia syndromes. These disorders usually result from interactions of three α -thalassaemia (α thal) genes; α thal 1, α thal 2 and Hb Constant Spring. In many human populations the α -chain loci are duplicated, that is, there are two per haploid genome². The gene α thal 1 results from a deletion of both of the pair of haploid α -chain genes^{3,4}, α thal 2 from a deletion of one of the pair⁵, and Hb Constant Spring from a chain termination mutation affecting one of the linked loci which markedly reduces the output of α chains and produces a phenotype almost identical to α thal 2 (ref. 6). Haemoglobin H disease results from the inheritance of α thal 1 together with either α thal 2 or Hb Constant Spring. There have been occasional reports of an acquired form of Hb H disease occurring in association with leukaemia or related myeloproliferative disorders⁷⁻¹². We have studied the blood and bone marrow of a patient with acquired Hb H disease and we report here that it has a completely different molecular basis from the genetic form.

The patient (WD), an 81-yr-old British male, had a bizarre myeloproliferative disease which terminated in acute myeloblastic leukaemia. Full haematological and biochemical data will be presented elsewhere. His blood picture showed two cell populations; most of the cells were poorly haemoglobinised but about 5% seemed normal. Chromosomal analysis of the

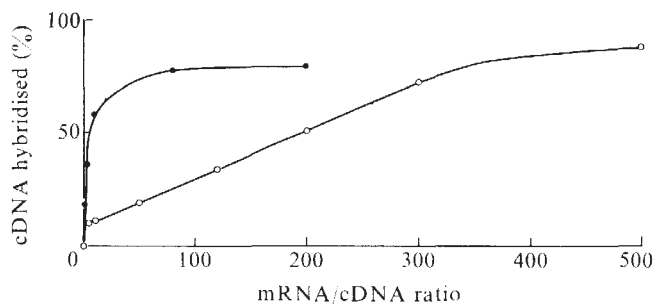


Fig. 1 Hybridisation of human globin cDNA α and cDNA β to mRNA from the peripheral blood of WD. cDNA α and cDNA β were prepared by the hybridisation of 7 μ g of $\beta^0/\delta^0\beta^0$ -thalassaemia mRNA to 190 ng of 3 H-cDNA $\alpha\beta$ (prepared as previously described¹⁰ by RNA-dependent DNA polymerase purified from avian myeloblastosis virus, a gift from Dr Beard, Life Sciences Research Laboratories) in 50 μ l of hybridisation buffer (0.5 M NaCl, 25 mM HEPES, 10 mM EDTA pH 6.8, 50% formamide and 500 μ g ml⁻¹ of *Escherichia coli* RNA) for 3 h at 43 °C. The non-hybridised component (cDNA β enriched) was separated from the hybridised component (cDNA α enriched) by hydroxylapatite fractionation at 60 °C. The cDNA α probe hybridised to mRNA $\alpha\beta$ to 90% completion and contained approximately 10% cDNA β as determined by hybridisation to mRNA lacking α -globin sequences. The cDNA β probe hybridised to 80% completion and contained 12% cDNA α as determined by hybridisation to mRNA lacking β -globin sequences. 9S mRNA from the peripheral blood of WD was prepared by phenol-chloroform extraction followed by sucrose gradient sedimentation. 0.1 ng of cDNA α (○) or cDNA β (●) were mixed with increasing amounts of mRNA in 2 μ l of hybridisation buffer in a sealed siliconised glass capillary tube and incubated at 43 °C for 100 h. The amount of cDNA hybridised was determined after incubation with S₁ nuclease¹⁹. The ratio of mRNA β to mRNA α is equal to the mRNA/cDNA ratio at which 50% of the cDNA α is hybridised divided by the ratio at which 50% of the cDNA β is hybridised. The cDNA concentration is corrected for the maximum amount of hybridisable cDNA. 50% of cDNA α was hybridised at ratio of approximately 200:1, and 50% of cDNA β at 3:1, giving a mRNA β /mRNA α ratio of 65:1.

peripheral blood and marrow was normal. Approximately 95% of the red cells generated typical Hb H inclusions and haemoglobin analysis showed 50-65% Hb H. The genetic form of Hb H disease was excluded because WD had been shown to have a normal blood picture 10 yr previously and examination of his children showed no evidence of thalassaemia or defective α -chain synthesis. The ratio of α - to β -chain synthesis in the peripheral blood was 0.07 as measured by 3 H-leucine incorporation^{13,14}. In the genetic form of Hb H disease this ratio ranges from 0.3 to 0.62 with a mean of 0.5^{15,16}.

mRNA prepared from the peripheral blood was translated in the wheat germ cell free protein synthesis system and the product analysed by CM-cellulose chromatography¹⁷. The mRNA directed the synthesis of β chains but no α -chain synthesis could be detected. In a similar study using mRNA from five patients with the genetic form of Hb H disease the α/β -chain production ratio ranged from 0.05 to 0.25 (mean 0.16)¹⁷. To determine whether the absence of α -chain synthesis was due to the absence of mRNA α or due to an abnormal mRNA α , mRNA was hybridised to purified complementary cDNAs as described previously¹⁸ (Fig. 1). At low mRNA/cDNA ratios the cDNA α probe hybridised to approximately 10%, which represents the hybridisation of contaminating cDNA β sequences. The cDNA α probe continued to hybridise at a slow linear rate up to the maximum level of 90% hybridisation at very high mRNA/cDNA ratios, indicating that a small amount of intact mRNA α sequences were present in the mRNA. The mRNA β /mRNA α ratio was calculated to be 65:1 and hence the amount of mRNA α relative to mRNA β was 1.5%.

To determine whether the virtual absence of mRNA α resulted from a transcriptional defect or a deletion of the α -globin genes in the malignant cell line, the hybridisation

kinetics of cDNA_α in cDNA excess with DNA from the erythroid bone marrow of WD were compared with those of normal DNA^{18,19}. Figure 2 shows that the cDNA_α probe gave a similar hybridisation curve with both DNA species indicating that α-globin sequences were present in the DNA to the same extent as normal DNA. But, the cDNA_α probe is less than half the length of the α-globin gene and thus a gene deletion beyond the region of DNA complementary to the cDNA_α probe would not be observed. To check that a gene deletion in the region of DNA complementary to the cDNA_α probe could be detected in our experimental conditions, cDNA_α was hybridised in cDNA excess to DNA prepared from the liver of an infant with the Hb Bart's hydrops syndrome^{3,4} and to a mixture of equal amounts of hydrops DNA and normal DNA (Fig. 2). The cDNA_α hybridised to the mixture to half the extent seen with normal DNA and hybridised to the hydrops DNA to a lesser extent than the mixed DNA, showing only a very small rise above the background value. The plateau value of the amount of cDNA hybridised to DNA in cDNA excess was used to calculate the number of globin genes hybridising to the cDNA_α. Table 1 lists the results for the hybridisation curves shown in Fig. 2, together with results of other cDNA excess hybridisations (not shown) conducted as control experiments. These results indicate that there is no major deletion of the α-chain genes in the DNA of the abnormal cell line of WD. Furthermore, they confirm previous observations^{3,4} that the α-globin gene sequences are largely deleted in the Hb Bart's hydrops syndrome, that is, the homozygous state for α thal 1. The observed small amount of hybridisation of hydrops DNA (0.2 genes) to the cDNA_α probe can be accounted for by contamination of the cDNA_α probe with about 10% cDNA_β.

The deficit of α-chain synthesis in this patient with acquired Hb H disease was much more marked than that which is found in the genetic form of the disease. Indeed, there was probably no α-chain synthesis in the neoplastic red cell line. Thus the pattern of haemoglobin synthesis was very similar to that observed in the Hb Bart's hydrops syndrome²¹ except that in this case

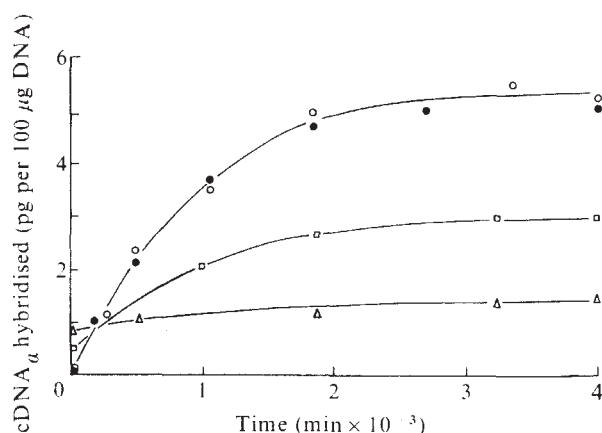


Fig. 2 Hybridisation of human globin cDNA_α in cDNA excess to DNA from the bone marrow of WD, DNA from normal human spleen and DNA from Hb Bart's hydrops foetalis liver. DNA was prepared from cell nuclei by hydroxylapatite chromatography²¹ and sonicated to 200–300 nucleotides in length. cDNA_α 20 pg was mixed with 100 μg of DNA in 20 μl of 0.12 M sodium phosphate, pH 6.8 and sealed in a siliconised glass capillary tube. The mixtures were denatured for 10 min at 100 °C and annealed at 66 °C. At the appropriate times, the amount of cDNA hybridised was determined after incubation with S₁ nuclease¹⁹. The percentage of cDNA hybridised was converted to pg of cDNA hybridised using the formula described by Bishop and Freeman²⁰. The cDNA concentration is corrected for the maximum amount of hybridisable cDNA. The figure shows the hybridisation of cDNA_α in cDNA excess to DNA from the bone marrow of WD (●), normal DNA (○), hydrops DNA (△) and a mixture (■) of equal amounts of normal DNA and hydrops DNA.

Table 1 No. of globin genes per haploid genome as determined by cDNA excess hybridisations

cDNA	DNA	cDNA hybridised (pg)	Gene complexity	Gene no.
cDNA _α	WD, blood	5.0	90,000	1.5
cDNA _α	WD, bone marrow	5.6	101,000	1.6
cDNA _α	Normal	5.4	97,000	1.6
cDNA _α	Normal/hydrops	2.4	43,000	0.8
cDNA _α	Hydrops	0.5	9,000	0.2
cDNA _β	WD, Blood	7.2	130,000	1.9
cDNA _β	WD, Bone marrow	7.6	137,000	2.0
cDNA _β	Normal	8.1	146,000	2.1

cDNA excess hybridisations were performed as described in Fig. 2 legend. The gene complexity is calculated from the plateau value pg of cDNA hybridised per 100 μg DNA using the formula described by Bishop and Freeman²⁰. The gene number is equal to the gene complexity divided by the cDNA complexity (60,000 for cDNA_α, 70,000 for cDNA_β).

β-chains were being produced instead of γ chains. Furthermore, unlike the Hb Bart's hydrops syndrome, the α-globin genes were intact in the neoplastic cell line in which α-chain synthesis was abolished. These findings suggest that there must have been a defect in α-chain gene transcription affecting both sets of haploid α-chain genes. One possible explanation is that there are specific repressors of the α-chain loci which are normally active only in embryonic life before α-chain synthesis commences, and that these repressors had been activated in the leukaemic cell line. This seems to be the most likely type of mechanism which could produce a *cis-trans* effect as evidenced by the suppression of both pairs of α-chain genes. There are examples of reversion to foetal erythropoiesis in leukaemia²² although regression to an earlier stage has not been reported. The lack of synthesis of embryonic ζ and ε chains in the abnormal cell line is incompatible with there having been a complete reversion to embryonic haemoglobin synthesis; the genetic mechanism which inhibited α-gene transcription was highly specific for the α-chain loci.

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Polymerisation of haemoglobin SA hybrid tetramers

ERYTHROCYTE sickling and gelation of concentrated solutions of deoxyhaemoglobin (Hb) S ($\alpha_2\beta_2^{6Val}$) results from helical polymerisation of the tetramers, with a spatial orientation approximated by recent ultrastructural and optical studies. Earlier observations of the gelling behaviour of Hb S or Hb C Harlem ($\alpha_2\beta_2^{6Val, 73Asn}$) mixed with Hb A or other haemoglobins gave indirect evidence concerning intertetrameric contact sites, and we proposed that only one $\beta 6$ valine-determined site was active per tetramer, the other β chain providing different polymer contacts^{1,2}. Our arguments required that asymmetrical hybrids (for example $\alpha_2\beta^A\beta^S$) occurred in Hb mixtures; their long-suspected presence has recently been established³⁻⁵, but the dissociation equilibria by which these hybrids form (Fig. 1) hinders their isolation and direct testing of their ability to polymerise. To circumvent this difficulty we have prepared Hb SA hybrids cross-linked intratetramERICALLY to prevent dissociation, and have found them capable of polymer and gel formation quite similar to that of Hb S.

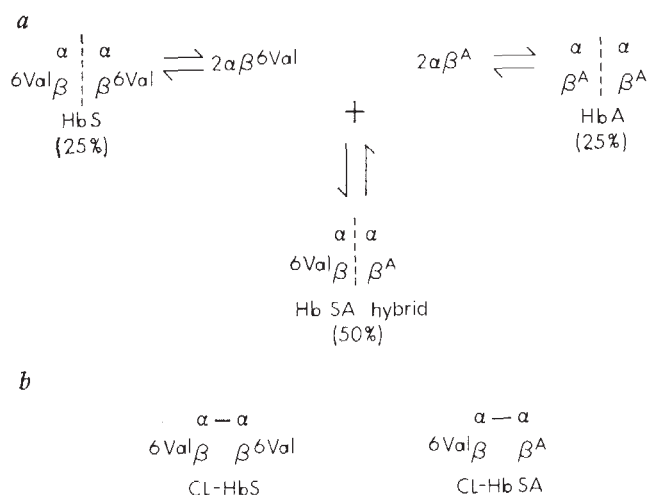


Fig. 1 Comparison of hybrid tetramer formation with cross-linked haemoglobins. *a*, Formation and distribution at equilibrium of hybrid and non-hybrid tetramers in a 1:1 mixture of Hb S and Hb A. *b*, Cross-linked Hb S and Hb SA hybrids as prepared in the present experiments; the covalent cross-link between the α -chain amino termini prevents dissociation into $\alpha\beta$ dimers, stabilising the SA hybrid.

Haemoglobin S and mixtures of equimolar amounts of Hb S and Hb A were each reacted with the bifunctional cross-linking reagent, difluorodinitrophenylsulphone, as described by Macleod and Hill⁶, and the cross-linked tetramers were separated from unreacted haemoglobin by chromatography on Sephadex G-100 in 1 M $MgCl_2$. Isoelectric focusing of the cross-linked tetramer fraction of reacted Hb S alone showed only one major haemoglobin band, whereas the corresponding tetramer fraction from the Hb S-Hb A reaction mixture showed three distinct bands which were stable in the liganded (CO) state. One of these three bands corresponded in position to the cross-linked (CL-) Hb S, another to CL-Hb A; midway between those two was a more prominent band, not seen with the fractions from Hb S or Hb A alone, which represented the hybrid CL-Hb SA ($\alpha_2\beta^S\beta^A$). Preparative isoelectric focusing permitted complete separation of the bands of interest (Macleod and Hill had obtained three subfractions by ion-exchange chromatography of the tetramer fraction of their Hb SA reaction mixture, and they identified the middle fraction as a true S-A hybrid⁴. We observed (as they did) incomplete separation of the three fractions by their method; we therefore used isoelectric focusing

on a preparative scale, which achieved complete separation of the three fractions with the corresponding charge differences. Migrating anodal to the well defined bands on each gel was a diffuse zone of Hb which probably represented a variety of other cross-linked reaction products⁶). Although the corresponding subfractions were shown by Macleod and Hill to be heterogeneous, including tetramers with unidentified cross-links between $\alpha\beta$ dimers together with the major product cross-linked between the α -chain amino termini, our present experiments required only the isolation of a stable non-dissociating $\alpha_2\beta^A\beta^S$ fraction free from $\alpha_2\beta_2^S$. (Some CL-Hb S was obtained from the major band of focused gels for which the starting material was Hb S alone rather than the Hb S-Hb A mixture. As noted, this major band coincided with the cathodal band on gels of the S-A mixtures. After the gelling experiments the CL-Hb S and CL-Hb SA hybrids were re-examined by isoelectric focusing each alone and both mixed together. Although all the steps in handling resulted in some charge-heterogeneity in each sample, none of the CL-Hb S bands co-focused with the CL-Hb SA bands, confirming the absence of contamination of CL-Hb SA by any detectable form of $\alpha_2\beta_2^S$.) The gel bands were sliced and the Hb fractions, eluted by the technique of Suzuki *et al.*⁷, and stored in the CO-form in liquid N_2 . When sufficient material was accumulated, the CL-Hb S and CL-Hb SA were converted from the CO-form to the cyanmet form (to permit rapid conversion by dithionite into deoxy Hb) and minimum gelling concentrations (MGCs) were determined in duplicate, as previously described¹ with the following minor modifications: 300- μ l aliquots of Hb solution were equilibrated with N_2 in 5 ml Ehrlenmeyer flasks, and the cyanmethaemoglobin was converted to the deoxy form by anaerobic addition of sodium dithionite (2 mol per mol total haem). Both the CL-Hb S and CL-Hb SA gelled, liquified on chilling the flask in an ice bath and gelled on subsequent re-warming to room temperature, as occurs with native Hb S. The MGC values and other details of the experimental conditions are shown in Table 1. Following gelation the chilled duplicate samples were combined and transferred anaerobically to centrifuge tubes under oil, allowed to gel again by equilibration at room temperature and centrifuged at 25 °C for 120 min at 122,000g (in a Beckman L3-50 centrifuge using an SW65K rotor) to sediment the solid phase. The supernatant concentrations, representing polymer solubility⁸, were 25.6 g Hb dl⁻¹ for CL-Hb S and 28.7 g Hb dl⁻¹ for CL-Hb SA. The supernatants were decanted and portions of the packed solid phases were fixed in deoxygenated solutions of 3% glutaraldehyde. After osmication and dehydration, the pellets were embedded in Epon 812. Examination of ultrathin sections by electron microscopy revealed 'polymer' rods in the solid phases of both CL Hb S and CL-Hb SA which were irregularly distributed in all directions, measured 165-175 Å in diameter, and were indistinguishable from those seen by us and others in sections of deoxygenated sickle (SS) cells.

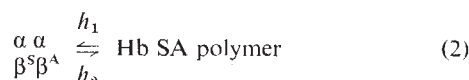
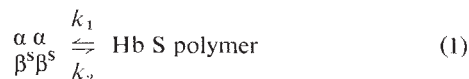
These findings show that the hybrid tetramers $\alpha_2\beta^S\beta^A$ are capable of gelation and polymerisation which is similar or identical to that of Hb S. Conformational changes in the cross-linked Hb, as indicated by its abnormal respiratory functions (high oxygen affinity and loss of cooperativity in oxygen binding)⁶ must account for the higher MGC value for CL Hb S as compared with native Hb S. The CL-Hb S may, nevertheless, serve as a control for comparison with the gelling behaviour of the CL-Hb SA. Having established that only one

Table 1 MGC values of cross-linked Hb SA hybrids compared with cross-linked and native Hb S

Native Hb S ($\alpha_2\beta_2^S$)	22.2
CL-Hb S	27.3
CL-Hb SA ($\alpha_2\beta^S\beta^A$)	30.8

Haemoglobin dialysed against 0.15 M potassium phosphate, pH 7.35, deoxygenated with N_2 gas at 25 °C, sodium dithionite added; final pH of Hb 6.9-7.0; mean MGC values expressed in g Hb dl⁻¹.

β^S chain per tetramer is required for polymerisation, we predict that Hb SA hybrids would have a smaller tendency to polymerise than Hb S tetramers. Compared with Hb S, which should be capable of fitting into the helical polymer in either of two symmetrical orientations (with either β^S chain providing the intermolecular binding site determined by the $\beta 6$ valine substitution), the SA hybrids would be expected to exhibit some steric hindrance for entry into the polymer, resulting in $k_1 > h_1$ in the following equilibria



There is reason to believe that the non-S β chain (or non-'active' β^S chain) of a tetramer provides other contacts in the polymer involving the regions of $\beta 73$ (E 17) and $\beta 121$ (GH4), but not the N-terminal region^{9,10} so that the forces maintaining the Hb S and Hb SA polymers, reflected in k_2 and h_2 , may be similar. In this case, the differences we have observed in both the MGC and polymer solubility values for CL-Hb S and CL-Hb SA reflect the expected differences in k_1 and h_1 described above.

By mixing haemoglobins in the deoxy state or by covalently cross-linking Hb A or Hb F tetramers (by the methods of Macleod and Hill) to prevent hybrid formation in Hb mixtures, we have previously shown that the well known inhibitory effect of Hb F on gelation of Hb S requires formation of $\alpha_2\beta^S\gamma$ hybrid tetramers; in contrast, the presence or absence of hybrid formation in mixtures of Hb S with Hb A had no net effect on the minimum gelling concentration¹¹. Similar results and conclusions were subsequently described by Goldberg *et al.*¹² who centrifuged the gels as an assay of polymer solubility. The differences in MGC and polymer solubility values of CL-Hb S and CL-Hb SA observed in our study are qualitatively consistent with our proposed explanation for the equivalent gelling tendencies of mixtures of Hb S and Hb A in which hybrid formation was permitted or prevented¹¹.

It is not yet clear whether or not Hb tetramers containing no β^S chains, such as Hb A or Hb F, are capable of entering the polymer. Our previous results suggested that these non-S tetramers facilitated gelling in a less specific manner¹¹ but the available evidence concerning this issue is quite indirect, and partially conflicting¹². Our results provide direct evidence that Hb SA hybrids can enter the polymer in place of Hb S tetramers, but with somewhat less efficiency than Hb S.

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The mechanism by which actinomycin D inhibits protein synthesis in animal cells

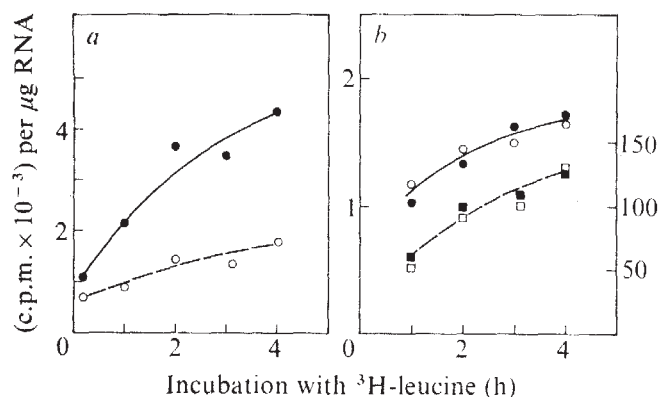
In the course of studies on the regulation of protein synthesis during the activation of human lymphocytes by phytohaemagglutinin (PHA), it became necessary to determine whether an effect which occurred following treatment with actinomycin D (AMD) was secondary to the action of the drug on transcription, or resulted from a direct effect on translation. Using enucleated lymphocytes, we have shown that AMD has no effect on protein synthesis in the absence of the cell nucleus and conclude that AMD interferes with protein synthesis by its effect on transcription of RNA.

AMD is a potent inhibitor of RNA synthesis in eukaryotic cells¹ and is widely used by molecular biologists. Protein synthesis diminishes in cells treated with AMD (refs 2, 3) and this was originally attributed to decay of messenger RNA (mRNA), but more recent findings have made that view untenable⁴⁻⁷. An additional effect of the drug, at the level of initiation of protein synthesis, has therefore been proposed, although the mechanism of this putative action has not been elucidated^{4,5}. In particular, it remains uncertain whether AMD inhibits protein synthesis by preventing the synthesis of some RNA component, other than mRNA, or by a direct action on the initiation of translation. Because of this uncertainty, the interpretation of much published data is subject to doubt.

To clarify this question, lymphocytes were enucleated by treatment with cytochalasin B followed by centrifugation in a density gradient as described by Wigler and Weinstein⁸. Lymphocyte cytoplasts produced in this way continued to incorporate ³H-leucine into acid-insoluble material for several hours (Fig. 1a). Since this incorporation was inhibited by puromycin (Fig. 1a), the action of which requires peptide bond formation⁹, we conclude that protein synthesis was occurring in cytoplasts by the normal mechanism. Cycloheximide, which inhibits protein synthesis by a different mechanism¹⁰, was also effective (results not shown). Cytoplasts prepared from lymphocytes previously stimulated by PHA for 16 h showed a ninefold higher rate of incorporation of amino acids than those from resting cells, reflecting the difference in protein synthetic activity commonly seen between resting and growing lymphocytes (Fig. 1b).

Addition of AMD to cytoplasts from either resting or growing lymphocytes had no effect on protein synthesis (Fig. 1b). Treatment of intact lymphocytes, either resting or growing, with AMD caused a diminution of protein synthesis, with progressive reduction in the incorporation of amino acids (Fig. 2a). Treatment of intact cells with cytochalasin B, in a manner similar to that used for producing cytoplasts, did not itself interfere with protein synthesis, nor did it prevent the inhibition of protein synthesis by AMD (Fig. 2b).

Inhibition of protein synthesis by AMD in resting and growing lymphocytes thus only occurs in nucleated cells. If a direct effect of AMD on translation occurred, it should be detectable in cytoplasts as well. Since it was not, we conclude that AMD inhibits protein synthesis in lymphocytes as a consequence of its well known action on nuclear RNA synthesis, and not by any direct action on translation. If the only action of AMD were functionally to enucleate the cell with respect to RNA synthesis, it follows that its effect on protein synthesis should be equivalent to mechanical enucleation. This hypothesis was



The upper layer of cytoplasts was aspirated, diluted with MEM and sedimented at $1,000g$ for 15 min. Cytoplasts were resuspended in MEM containing $1/20$ th the usual concentration of amino acids plus 2% autologous plasma, and incubated (37°C) with ^3H -leucine (Schwarz-Mann, 50 Ci mmol^{-1} , $10 \mu\text{Ci ml}^{-1}$). At the times indicated, duplicate 1.0-ml samples were applied to GF/C filters moistened with phosphate-buffered saline (PBS) containing unlabelled leucine and washed first with PBS-leucine under gentle suction, then with cold 5% trichloroacetic acid-leucine. After drying, radioactivity remaining on the filter was measured by liquid scintillation counting. The RNA content of the suspensions was measured by precipitation with cold 2% perchloric acid (PCA) (10 min), washing with cold 2% PCA, digestion in 10% PCA at room temperature for 17 h, removal of insoluble material by centrifugation and measurement of A_{260} , using $E_{1\%}^{1\text{cm}} = 320$. Results were expressed as c.p.m. μg^{-1} RNA. No correction was made for radioactivity in leucyl-tRNA, as previous experiments showed the contribution from this source to be negligible. *a*, Effect of puromycin on amino acid incorporation: ●, untreated cytoplasts from resting lymphocytes; ○, $100 \mu\text{g ml}^{-1}$ puromycin added 5 min before addition of ^3H -leucine. *b*, Effect of actinomycin D on protein synthesis by cytoplasts: ●, resting-cell cytoplasts, no AMD; ○, resting-cell cytoplasts, $1 \text{ mg } \mu\text{l}^{-1}$ AMD added with ^3H -leucine. ■, Cytoplasts from lymphocytes incubated 16 h with PHA ($5 \mu\text{g ml}^{-1}$), no AMD; □, growing-cell cytoplasts, $1 \mu\text{g ml}^{-1}$ AMD added.

supported by a comparison between data from several experiments with cytoplasts and with AMD-treated intact cells (Fig. 2c). The two sets of data showed no significant difference in the kinetics which characterise the progressive fall in protein synthesis.

It has been reported previously that at least 50% of newly-synthesised mRNA (cytoplasmic RNA⁺ poly(A)) in resting lymphocytes is very short lived, with a half life of the order of 20 min, whereas the remainder of newly synthesised mRNA is highly stable, with a half life in excess of 20 h (ref. 11). Using this information, and assuming a minimum half life for stable mRNA of 20 h, it may be calculated that the steady-state level of labile mRNA in non-growing lymphocytes is on the order of 2% of the total, the remainder being highly stable. It is

likely, therefore, that AMD interferes with translation by abolishing the synthesis of some RNA species other than mRNA⁺ poly(A), since the small contribution of labile mRNA to the steady-state total would not account for the effect of the drug.

Lymphocyte protein synthesis is virtually unaffected by concentrations of AMD which specifically inhibit rRNA synthesis¹², and ribosomal 5S RNA synthesis is not abolished by such low concentrations of AMD, but its appearance in the cytoplasm is prevented¹³. Since this deficiency is not associated with diminished protein synthesis, it is evident that interruption in the supply of new 5S RNA to the cytoplasm is not the means by which higher concentrations of AMD inhibit protein synthesis. Craig³ dismissed reduced tRNA synthesis as a possible cause

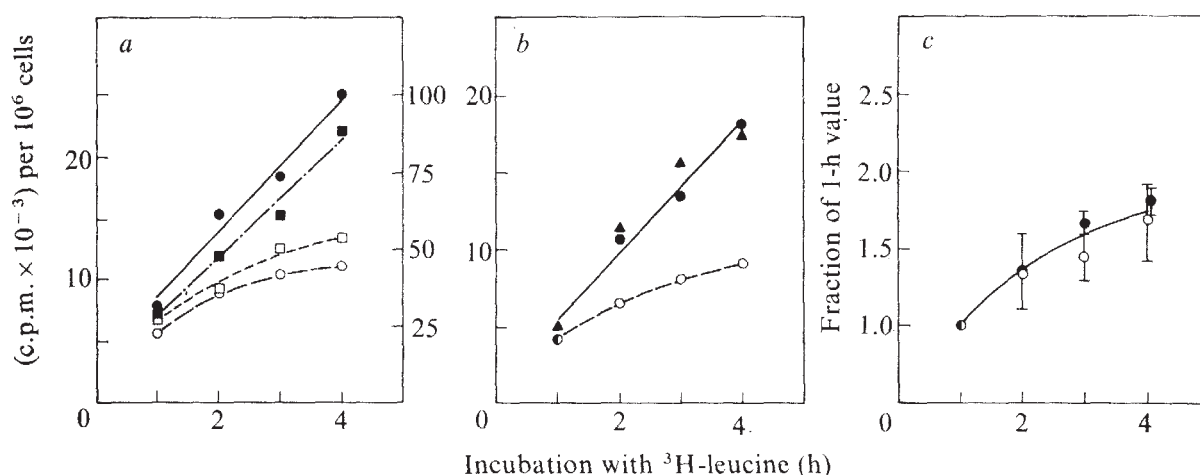


Fig. 2 Effect of actinomycin D on protein synthesis by intact lymphocytes. Lymphocytes were suspended at $2 \times 10^6 \text{ ml}^{-1}$ in MEM, $1/20$ th amino acid concentration, 2% autologous plasma and incubated with ^3H -leucine ($10 \mu\text{Ci ml}^{-1}$). Cell collection and analysis were as in Fig. 1. *a*, Effect of AMD on protein synthesis in resting and growing lymphocytes: ●, resting lymphocytes, no AMD; ○, resting lymphocytes, $1 \mu\text{g ml}^{-1}$ AMD added at zero time; ■, PHA, 16 h before labelling, no AMD; □, PHA, 16 h, AMD, $1 \mu\text{g ml}^{-1}$, added at zero time. *b*, Effect of cytochalasin pretreatment on protein synthesis by intact lymphocytes. Resting cells were incubated for 1 h with $10 \mu\text{g ml}^{-1}$ cytochalasin B in MEM, 10% autologous plasma, then centrifuged and resuspended in labelling medium. ●, No CB treatment; ▲, CB-treated cells; ○, CB-treated cells, $1 \mu\text{g ml}^{-1}$ AMD added with ^3H -leucine. *c*, Comparison of kinetics of amino acid incorporation in resting-cell cytoplasts with those of intact resting cells treated with AMD. Results from three experiments with intact cells and four experiments with cytoplasts were normalised by expressing results as fraction of 1-h value. Mean and $\pm 2 \text{ s.e.m.}$ are given. ○, Cytoplasts; ●, AMD-treated resting cells.

for inhibition of protein synthesis in AMD-treated L cells because 8 h of exposure to $0.25 \mu\text{g ml}^{-1}$ AMD caused a 50% reduction in protein synthesis. This concentration of the drug was thought to be too low to inhibit tRNA synthesis. No actual data were given to demonstrate unimpeded tRNA synthesis, however, and in our view, tRNA has not been eliminated as the critical molecule whose synthesis, when interrupted by AMD, causes cessation of protein synthesis. This thesis is presently being examined, together with the more general proposition that availability of tRNA may have a regulatory role in governing the rate of protein synthesis in resting lymphocytes.

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Regulation of maternal mRNA translation in developing embryos of the surf clam *Spisula solidissima*

MESSANGER RNA not bound to ribosomes (non-polysomal mRNA) has been found in the cytoplasm of sea urchin^{1–3} and *Spisula solidissima* eggs⁴. The mRNA synthesised and stored during oogenesis (maternal mRNA) is utilised when protein synthesis is activated by fertilisation. Translation of maternal mRNA allows sea urchin embryos to proceed through several cellular divisions even when synthesis of embryonic mRNA is blocked by actinomycin D^{5–8}. But, embryonic mRNA is synthesised soon after fertilisation and already translated at the early cleavage stage⁷. Qualitative and quantitative changes in the pattern of histone synthesis during sea urchin embryogenesis have suggested that the synthesis of these proteins is temporally regulated in the embryo and that different mRNA species are translated at successive stages of embryo development⁸. We report here that part of the mRNA is found unassociated with ribosomes in eight-cell embryos of *S. solidissima*. Some of the translation products of this non-polysomal mRNA are different from those of polysomal mRNA, but identical to translation products of maternal mRNA isolated from eggs. This suggests that part of the non-polysomal mRNA of embryos consists of maternal mRNA species which are inefficiently translated after fertilisation.

Cytoplasmic extracts of *S. solidissima* embryos were fractionated by centrifugation on sucrose gradients (Fig. 1). The RNA isolated from the polysome pellet and from gradient fractions was translated in the wheat germ cell-free system⁹. Polysomal RNA and the RNA extracted from a cytoplasmic fraction sedimenting between 20S and 60S (non-polysomal mRNA) significantly stimulated protein synthesis and were

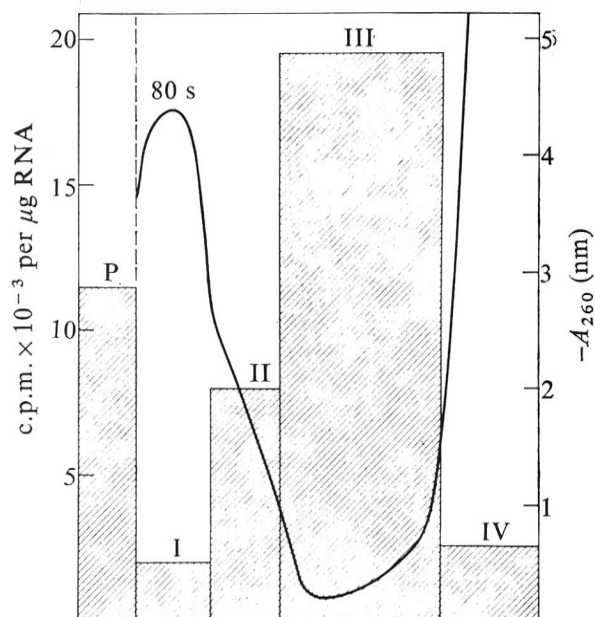


Fig. 1 Fractionation of *S. solidissima* eight-cell embryos homogenate by centrifugation on sucrose gradient. Eggs were collected, washed and fertilised⁴ and embryos were collected by centrifugation and washed as described before⁴. One volume of packed embryos was homogenised with 4 volumes of TNM (20 mM Tris-HCl pH 7.6, 0.2 M NaCl and 5 mM magnesium acetate). The homogenate was centrifuged for 10 min at 12,000 r.p.m. and 4.5 ml of the supernatant were fractionated by 19 h centrifugation at 26,000 r.p.m. through a 34-ml 20–40% sucrose gradient in TNM. The gradients were monitored for A_{260} (solid line) and subdivided into four fractions. Polysomes were recovered in the pellet. RNA was obtained from gradient fractions and from the polysome pellet by phenol extraction and ethanol precipitation¹⁷. The RNA was assayed in a wheat germ cell-free system prepared according to Roberts and Patterson⁹. Each fraction was tested at several RNA concentrations and the stimulation of protein synthesis measured by the incorporation of ³H-lysine into protein in 60-min incubations as previously described¹⁷. The activity of each fraction was calculated from a range of concentrations which gave a stimulation of protein synthesis proportional to the input of RNA. The activity of each fraction is expressed as c.p.m. $\times 10^{-3}$ per μg RNA. Twenty mg of RNA were recovered from the polysome pellet and 5.3 were recovered from fraction III per ml of packed embryos fractionated. Taking into account the relative stimulatory activity and the amount of RNA recovered, it was calculated that the total template activity of polysomal RNA was four times that of fraction III RNA.

directly used in subsequent experiments. About 80% of the template activity of embryo RNA was found in the polysome pellet, and the remaining 20% was found in the fraction sedimenting at 20–60S (Fig. 1).

The translation products of polysomal and non-polysomal embryo mRNA were compared with those of mRNA obtained from whole eggs and purified by absorption of oligo (dT)-cellulose¹⁰. The translation products were fractionated by electrophoresis on polyacrylamide gels containing sodium dodecylsulphate (SDS)¹¹ and detected by fluorography¹² (Fig. 2). The translation products of embryo polysomal mRNA correspond predominantly to marker histones. But, the products of embryo non-polysomal mRNA show in addition to histones some bands which are not detected among the translation products of polysomal mRNA (indicated by arrows in Fig. 2). The translation products of egg mRNA show two bands corresponding to histones H1, several poorly resolved bands migrating with or slightly faster than marker histones, and other bands corresponding to translation products of both polysomal and non-polysomal embryo mRNA. Aliquots of each translation assay were extracted with 0.2 M H_2SO_4 (ref. 13) to further purify histones. The proteins extracted with acid were fractionated by SDS gel electrophoresis (Fig. 2). This analysis showed that

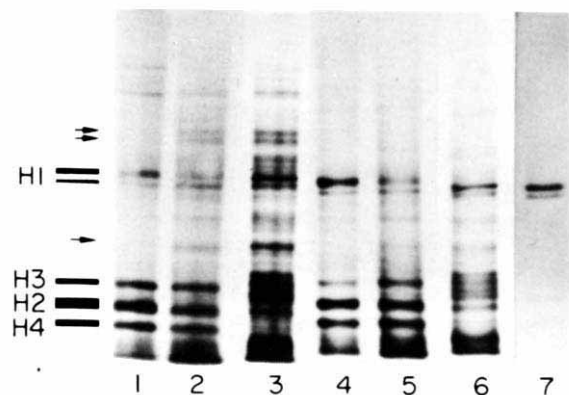


Fig. 2 Analysis of the translation products of polysomal and non-polysomal embryo RNA and of egg RNA. Embryo RNA was prepared as described in Fig. 1. RNA was obtained from eggs homogenised with 4 volumes of TNM, centrifuged 10 min at 12,000 r.p.m., made 1% in SDS and extracted with phenol¹⁷. The RNA was chromatographed on oligo(dT)-cellulose¹⁰ and the poly(A)-containing fraction used for translation assays. The RNA was translated with wheat germ extract containing 0.88 mCi ml⁻¹ of ³H-lysine (40 Ci mol⁻¹). Aliquots of each incubation were analysed by electrophoresis on 12.5% polyacrylamide gels containing SDS¹¹. The radioactive proteins were detected by fluorography of the dried gels¹². The position of marker histones prepared from developing embryos⁴ is shown schematically on the left. Track 1, translation products of polysomal embryo RNA; 2, translation products of fraction III non-polysomal RNA (see Fig. 1); track 3, translation products of egg RNA. An aliquot of each assay was treated with 0.2 M H₂SO₄ to extract the acid-soluble proteins¹³, which were then precipitated with 4 volumes of ethanol and analysed in parallel. Tracks 4, 5 and 6, acid-soluble proteins prepared from the translation assays analysed in tracks 1, 2 and 3, respectively. Track 7, marker H1 histones purified from developing eight-cell embryos incubated with ¹⁴C-lysine⁴; the H1 histones were isolated by precipitating the other histones with 5% perchloric acid²¹. The arrows on the left indicate bands present among the translation products of non-polysomal embryo RNA and of egg RNA.

all the embryo and egg peptides co-migrating with marker histones are acid extractable. Among the egg mRNA translation products, histones H1 are clearly identified, whereas other histones are present in relatively lower amounts. In subsequent experiments egg and embryo RNA were fractionated in parallel into size classes by centrifugation on sucrose gradients (Fig. 3). The RNA sedimenting between 4 and 18S, which is enriched in histone mRNA¹, was isolated and translated in the wheat germ cell-free system. The translation products of 4–18S RNA were analysed by electrophoresis in a gel system devised for the fractionation of histones, which separates proteins primarily on the basis of their charge¹⁴. The two H1 histones, which separate in SDS gel electrophoresis, migrate together in this gel system (Fig. 3). The translation product of 4–18S egg RNA contain H1 histone and two additional peptides, which correspond to translation products of embryo mRNA. One peptide migrates like H4 histone and the other between marker H2A and H1 histones. This peptide also appears among the translation products of embryo non-polysomal mRNA, but not among the products of embryo 4–18S polysomal mRNA (Fig. 3). This peptide represents one further example of a maternal template which is found among non-polysomal mRNA species in the embryo.

The translation products of egg mRNA isolated by oligo(dT)-cellulose chromatography were also analysed in the gel system described above (Fig. 3). By overloading the gel it was possible to show that egg RNA codes for the other histones in addition to H1 histones. This suggests that mRNAs for all histones are present in *S. solidissima* eggs, though in quite different relative amounts, and that these mRNAs may contain poly(A) since they bind to oligo(dT)-cellulose. Histone mRNA obtained from *Xenopus*¹³ and *Triturus*¹⁵ eggs contain poly(A), whereas histone mRNA

obtained from mammalian cells does not contain poly(A)¹⁵ and does not bind to oligo(dT)-cellulose¹⁰.

The analysis of mRNA distribution in eight-cell embryos of *S. solidissima* shows that part of the mRNA is not associated with polysomes and therefore is not actively translated at this stage of development. We have only examined in detail the RNA distribution in eight-cell embryos. In older embryos there is less non-polysomal mRNA (our unpublished observations), whereas in eggs most of the mRNA is non-polysomal⁴. A simple interpretation of these observations is that during embryonic development maternal mRNA is translated in an orderly fashion. In the eight-cell embryo some mRNA species are preferentially translated, whereas others are not translated and are therefore found as non-polysomal mRNA species. We have no evidence that these non-polysomal mRNA species are translated later in embryogenesis, though this seems in view of the decrease in non-polysomal mRNA. We suggest, however, that the presence of non-polysomal mRNA is due to translational regulation in eight-cell embryos. Translation of maternal and newly synthesised mRNAs may be regulated in a specific way. This regulation does not involve the activation of maternal templates by addition of the 5'-terminus m⁷Gppp (ref. 17), since *S. solidissima* egg mRNAs already contain this structure (unpublished).

Various mechanisms have been proposed to explain translational regulation (see ref. 18 for review). These mechanisms can be subdivided into two types: (1) regulation involves a change in the components of the translational machinery; (2) regulation results from changes in the species of mRNA present and/or in the activity of the cell to initiate synthesis of new polypeptides. Our experiments have not been directed at studying the mechanisms of translational regulation operating in embryos. An analysis of the events which take place on fertilisation makes it possible, however, to propose an interpretation of the regulatory pattern observed.

The predominant templates translated by embryos are histone mRNAs (Fig. 1). These templates are present in relatively small amounts among maternal mRNA species, with the exception of H1 histone mRNA⁴. Active synthesis of histone mRNA on fertilisation is necessary to account

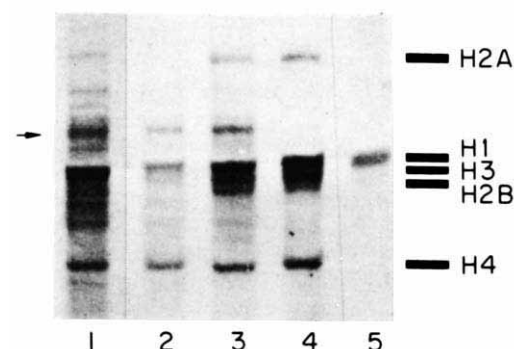


Fig. 3 Analysis of the translation products of embryo and egg RNA. The translation assays are described in Figs 1 and 2. Proteins were fractionated on 12% polyacrylamide gels containing 7.5 M urea, 6 mM Triton X-100, and 0.9 M acetic acid¹⁴. The proteins were detected by fluorography¹². Tracks 1 and 3, translation products of egg RNA containing poly(A) and of fraction III non-polysomal embryo RNA (see Fig. 1). The egg RNA and polysomal embryo RNA were fractionated by centrifugation on sucrose gradients^{1,17} and the fractions containing RNA sedimenting between 4S and 18S pooled. Track 2, translation products of 4–18S egg RNA; track 4, translation products of 4–18S polysomal embryo RNA. Track 5, marker H1 histones prepared as described in Fig. 2. The arrow at the right indicates a prominent band which is found among the translation products of egg 4–18S and of embryo non-polysomal RNA. The position of marker histones, prepared from developing embryos⁴ is indicated on the right.

for the synthesis of histones in embryos. Synthesis of mRNA with the sedimentation characteristics of histone mRNA has indeed been reported in *S. solidissima* embryos¹⁹. The presence of histone mRNA among the non-polysomal mRNA species (Fig. 2) suggests that the supply of mRNA exceeds the capacity of the eight-cell embryos to translate this mRNA. This situation leads to competition among different mRNA species for translation. Histone mRNA is one of the 'strongest' mRNA molecules. Treatments of HeLa cells which result in competition among mRNAs for translation suppress the synthesis of most cellular proteins except histone²⁰. The translational regulation observed in *S. solidissima* embryos may be based on a similar mechanism. Initiation may be the limiting step in protein synthesis in embryos and this may lead to competition among different mRNAs for translation. Histone mRNA may therefore be preferentially translated over other 'weaker' mRNAs, which are presumably translated later in development, when initiation is no longer limiting. This regulatory model does not involve any qualitative change in the components of the translational machinery, but only an increase in the activity of embryos to initiate protein synthesis.

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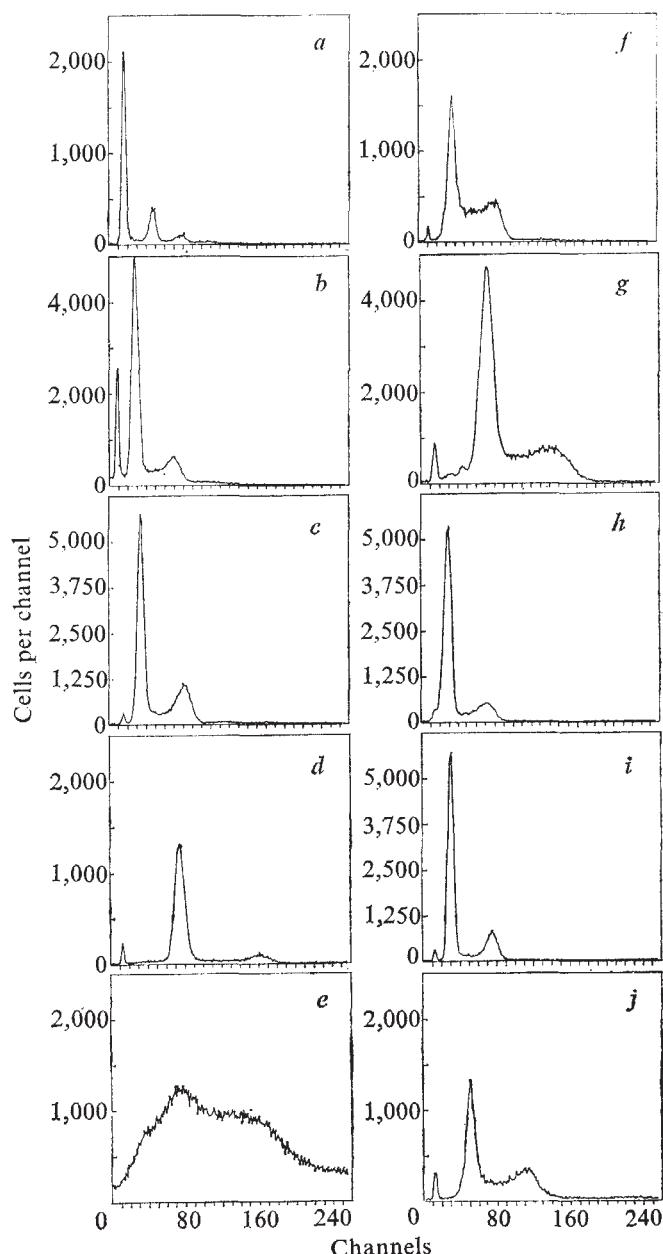
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DNA content of clones isolated from a fibrosarcoma. All the clones examined had a higher DNA content than did either the normal cells tested or the original fibrosarcoma. There is thus a variability of DNA content in fibrosarcoma cells. This suggests that variability, as well as increase in DNA content in malignant cells may relate to their neoplastic development.

The clones FSA 1231, 1233, 1235, 12310, 12313, 12314, 12316, 12317, and 12318 were isolated from a methycholanthrene-induced fibrosarcoma by repeated cloning in soft agar medium¹¹ under selective pressure for enhanced clonogenicity (N.S. and H.R.W. *J. natn. Cancer Inst.* in the press). The surviving fraction of the original tumour cells was 10^{-7} to 10^{-6} , compared with 0.1 to 0.5 for the resulting highly clonogenic cells. The cells were cultured in a humidified CO₂ incubator with McCoy's 5A medium as modified by Hsu and containing 20% foetal calf serum.

The method of FMF analysis has been described elsewhere¹²⁻¹⁴. Briefly, 2 to 5×10^6 exponentially growing cells or

Fig. 1 Histogram of DNA content distribution obtained using flow microfluorometry. FSA 1231 (c), 1233 (d), 12310 (e), 12313 (f), 12314 (g), 12316 (h), 12317 (i), 12318 (j) were added with normal spleen cells. The spleen peak of FSA 12316 is seen only as a notch, since it includes only a small amount of spleen cells. This was done to avoid an effect on the G₁ peak of the sample. a, Spleen cells; b, FSA original cells.



Variability of DNA content of murine fibrosarcoma cells

ANEUPLOIDY or heteroploidy and hyperploidy have been observed in tumours of experimental animals and of humans and seem to be a feature of most malignant cells¹⁻⁹. DNA contents seem to correlate with karyotype in these changes or deviations from euploidy. Most tumours are hyperploid and higher in DNA content^{2,8,9}. A rapid and reliable method of analysis of DNA content distribution of a cell population has recently become available with the development of flow microfluorometry (FMF)¹⁰. We report here the use of FMF analysis to measure

tumour cells were fixed with 70% ethanol and stained with mithramycin (Pfizer) for DNA. The staining solution contained 50 $\mu\text{g ml}^{-1}$ mithramycin and 7.5 mM MgCl_2 in 12.5% aqueous ethanol. The flow microfluorometer was of the type described by Steinkamp *et al.*¹⁰

Quantitation and comparison of DNA content were carried out by adding spleen cell suspensions to each sample. The spleen cell suspensions were made from normal untreated $\text{C}_3\text{Hf/Bu}$ mice from the specific pathogen-free breeding colony maintained at the M. D. Anderson Hospital, Section of Experimental Radiotherapy. The minced spleen tissues were filtered through a stainless steel mesh (200 wires per inch) screen and washed by centrifugation. The suspension included singlet, doublet and triplet aggregates of spleen cells (Fig. 1). When the relative DNA contents for singlets, doublets and triplets were assigned values of 1, 2 and 3, and were plotted on a linear scale against channel number, a straight line relationship was observed. The line extrapolated across the abscissa at a certain point. This intercept value (channel number) was used to correct zero offset.

Figure 1 represents typical histograms of DNA content in various clones. Spleen cells were added as a standard to each sample except that from the original fibrosarcoma (tumour cell suspension) and FSA 12310. The position of the peak for normal cells in the original fibrosarcoma was not affected by addition of spleen cells although its height was increased, that is, DNA content of normal cells (G_1 peak) in a tumour was the same as that of spleen cells. Intrinsic normal cells were therefore used as a standard. G_1 peak positions of these clones were different from one another. Furthermore, FSA 12310 was completely heterogeneous with respect to DNA content.

Table 1 shows the relative DNA content (G_1 peak) of the various clones relative to spleen cells. These ratios were calculated from the modal G_1 peak positions after correction for zero offset. Modal peak positions were determined from digital records of cell number for each channel. Relative DNA content ranged between 1.49 and 3.07, while the relative DNA content of the original fibrosarcoma was 1.45. All clones so far examined have shown a relative DNA content greater than 1.0, that is, they contained more DNA than did normal cells. DNA content in all except FSA 12310 has remained stable through cell divisions after *in vitro* and *in vivo* growth suggesting a hereditary nature of the DNA content.

The coefficients of variation (c.v., 100 times the standard deviation divided by the mean) of G_1 peaks shown in Fig. 1, were 7.7 (FSA original), 6.8 (FSA 1231), 6.1 (FSA 1233), 8.8 (FSA 12313), 8.5 (FSA 12314), 9.7 (FSA 12316), 6.0 (FSA 12317), 7.3 (FSA 12318), and 5.8 ± 0.3 (spleen cells, $\pm$ s.e. of the mean of eight measurements). The c.v. values of G_1 peaks of the original fibrosarcoma and of these clones were relatively large compared with that of normal spleen cells. The difference between some clones (FSA 1233 and FSA 12317) and spleen cells is not obvious, however.

Kramer *et al.* have investigated DNA content distribution of various established cell lines *in vitro* using FMF^{15,16}. They used c.v. of the G_1 peak to evaluate the heterogeneity of DNA content distribution in cell populations. They reported that DNA content by modal peak position of malignant cells was higher than that of normal cells. Surprisingly, however, the c.v. of the G_1 peak was not greater for malignant than for normal cells, even if a heterogeneity in the karyotype was observed. Thus, they concluded that DNA content distributions of malignant cells were not heterogeneous even if the karyotypes were, and that a change in karyotype does not always reflect an alteration in the DNA content of the cell. This conclusion challenges the classical view of the genetic entity of the chromosome.

We examined, using freshly isolated clones from a fibrosarcoma, whether DNA content of malignant cells varies and/or is greater than that in normal cells. In summary, our results lead us to conclude that these clones from a mouse fibrosarcoma were heterogeneous in DNA content implying that the cells had the ability to vary DNA content. All the clones derived by selective cloning and examined thus far have had a higher DNA content than the original fibrosarcoma cells. The results concerning DNA

Table 1 Relative DNA content of G_1 peaks of various clones

Cells	Relative DNA content	Mean $\pm$ s.e.
FSA original	1.45	—
FSA 1231	1.61, 1.50	1.56 ± 0.05
FSA 1233	3.03, 3.10	3.07 ± 0.03
FSA 1235	1.80	—
FSA 12312	1.60	—
FSA 12313	1.93, 2.00, 1.90	1.94 ± 0.03
FSA 12314	2.84, 2.87, 2.80	2.84 ± 0.02
FSA 12316	1.50, 1.44, 1.52	1.49 ± 0.02
FSA 12317	1.62	—
FSA 12318	2.39	—

Relative DNA content is given relative to the spleen singlet peak (G_1) which was assigned a value of 1.

content from this system are consistent with the accumulated findings of aneuploidy and hyperploidy in karyotypes of malignant cells¹⁻⁷. Since higher cellular DNA content was found in clones selected for higher plating efficiency in adverse growth conditions *in vitro*, changes in ploidy during tumour progression¹⁻⁷ may reflect a similar selection process in the host.

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Use of coupled transcription and translation to study mRNA production by vaccinia cores

VACCINIA is a double-stranded DNA virus which replicates in the cytoplasm of animal cells. Soon after infection, the outer layers of the virion are removed to yield core particles¹ which make mRNA that is transcribed, capped² and polyadenylated³ by enzymes present in the cores. Later, the cores break down, DNA synthesis begins, and 'late' genes are expressed⁴. Cores can be made *in vitro* by treating virions with non-ionic detergent and mercapto-ethanol. When incubated with nucleoside triphosphates, these particles produce mRNA⁵ which can be translated in cell-free systems to yield authentic vaccinia early proteins^{3,6,7}. It has recently been suggested that this mRNA may be derived from large primary transcripts⁸ which are processed in a manner similar to that proposed for cellular mRNA^{9,10}. I have obtained evidence that early vaccinia

mRNA is made by monocistronic transcription *in vitro*, by comparing the relative sensitivity of the synthesis of individual messages to ultraviolet irradiation. Production of mRNA was assayed indirectly using a coupled cell-free transcription-translation system in which protein synthesis is dependent on added vaccinia cores.

The use of partial ultraviolet inactivation to study transcription depends on the observation that the thymine dimers produced by irradiation cause premature termination of transcription¹¹. Assuming that all DNA is equally susceptible to ultraviolet damage, the sensitivity of synthesis of a given mRNA is proportional to the distance between its promoter and the 3' end of the cistron. In the case of messages which are primary transcripts, the ultraviolet sensitivity should be directly proportional to the length of the mRNA. This would clearly not be the case if several mRNAs were transcribed in tandem from a single promoter. Polycistronic transcription of the latter kind has been detected by ultraviolet irradiation experiments in DNA bacteriophages^{12,13}, adenovirus¹⁴, and the negative strand RNA viruses, vesicular stomatitis virus (VSV)^{15,16} and Sendai¹⁷.

The coupled transcription-translation system used in these experiments is based on the nuclease-treated reticulocyte lysate described previously¹⁸. Fig. 1 shows that when vaccinia cores are added to this system at concentrations similar to those found in infected cells, rapid synthesis of RNA begins after a brief lag. Protein synthesis starts after a further 5–10 min and continues linearly for

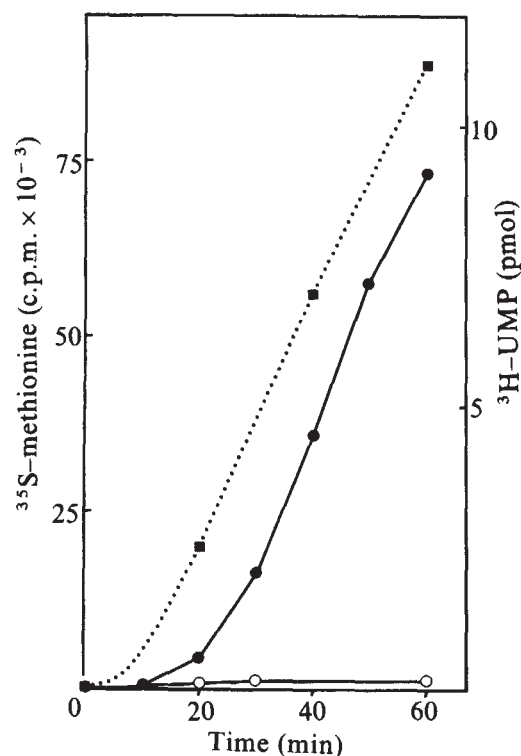


Fig. 1 RNA and protein synthesis directed by vaccinia virus cores in the nuclease-treated reticulocyte lysate. mRNA-dependent reticulocyte lysate prepared as described previously¹⁸ was supplemented by addition of 1 mM each of ATP, GTP, and CTP, 3.5 mM MgCl₂, 60 μg ml⁻¹ mouse liver tRNA, 2 mM glucose and either 20 μM methionine and 50 μM ³H-UTP (10.2 Ci mmol⁻¹) or 1 mM UTP and ³⁵S-methionine (4 × 10⁸ c.p.m. ml⁻¹; about 30 Ci mmol⁻¹ taking into account the unlabelled methionine present in the lysate). Cores, prepared as in ref. 5, were kindly provided by Jane Sykes, and were added at approximately 1.5 × 10¹⁰ ml⁻¹. Samples (5 μl) were removed at intervals for the assay of RNA synthesis by precipitation with cetyltrimethylammonium bromide (CTAB)²¹, or for protein synthesis as described previously¹⁸. Incubation temperature was 30 °C. ■, ³H-UTP incorporation; ●, ³⁵S-methionine incorporation; ○, ³⁵S-methionine incorporation in the presence of 7 μg ml⁻¹ actinomycin D.

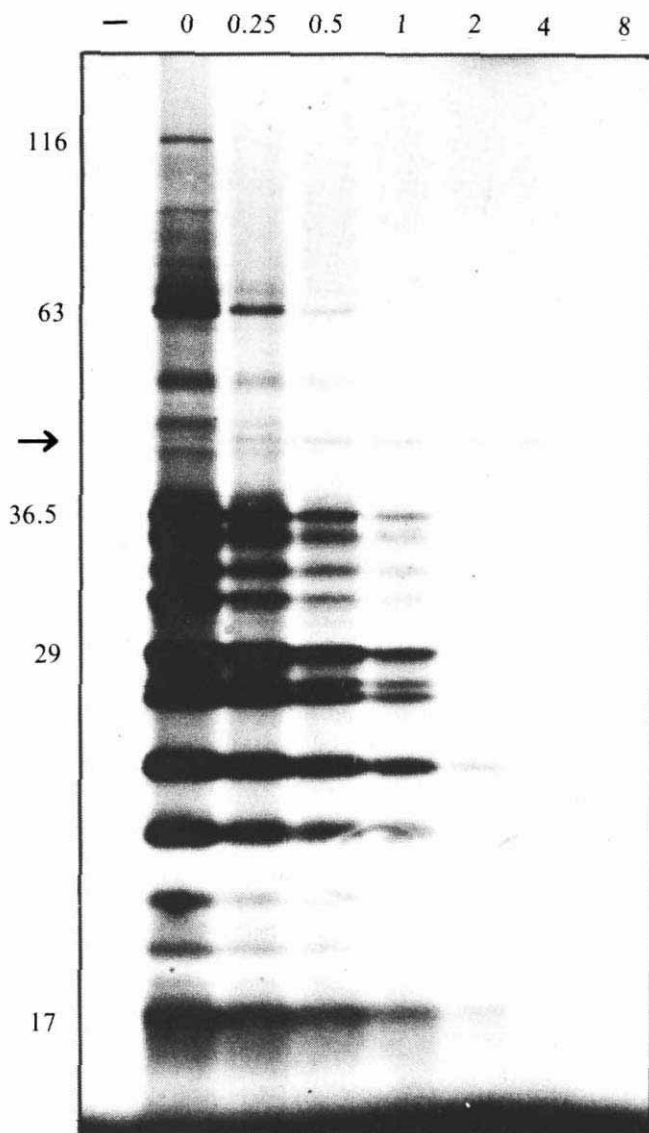


Fig. 2 Protein synthesis directed by ultraviolet-irradiated vaccinia cores. Drops (1 μl) containing (2–3) × 10⁸ vaccinia cores were placed on a sheet of parafilm and held on ice 30 cm from a 15-W germicidal ultraviolet lamp for various times. They were then added to 15 μl of the mixture described in Fig. 1, and incubated at 30 °C. Double-stranded RNA was present at a concentration of 20 μg ml⁻¹ to avoid inhibition of protein synthesis by low levels of endogenous double-stranded RNA present in the core preparations (see ref. 22). After 1 h of incubation, a further 10 μl of translation mixture was added, and the incubation continued for another 1 h. Samples (5 μl) were analysed on a 15% SDS-polyacrylamide gel. A fluorogram of this gel is shown. The molecular weights (× 10⁻³) indicated in the margin were derived by comparison with a set of ¹⁴C-iodoacetate-labelled marker proteins run in parallel with the experimental samples. Arrow indicates a background band which is labelled even in the absence of cores. The sample in the left-hand track contained no cores; others contained cores irradiated for the number of minutes indicated.

about 1 h. About 30–40 discrete polypeptides are made, many of which comigrate with authentic early vaccinia proteins on two-dimensional polyacrylamide gels of the O'Farrell type¹⁹ (data not shown). This protein synthesis is completely prevented by 7 μg ml⁻¹ actinomycin D, whereas the translation of globin or preformed vaccinia mRNA is unaffected by the drug. Protein synthesis in the coupled system is therefore dependent on the transcription of vaccinia DNA. Coupling RNA and protein synthesis in this way avoids the need for an intermediate purification of the RNA, thus eliminating errors due to unequal recovery of different messages. The direct study of

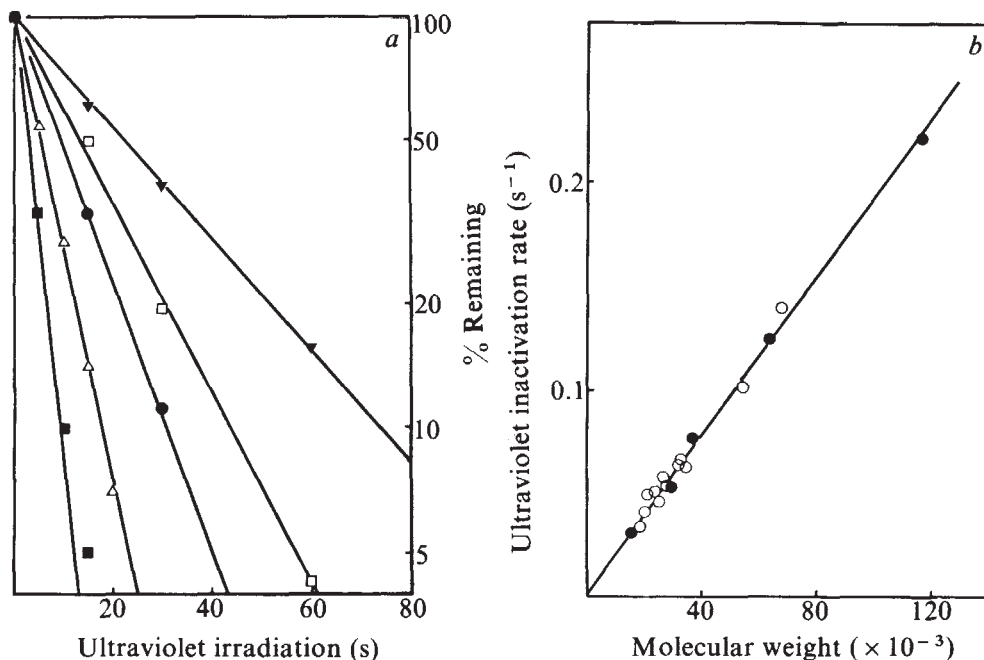


Fig. 3 Rate of ultraviolet light inactivation of synthesis of vaccinia proteins. *a*, Relative amount of radioactivity in five representative polypeptides plotted against the time of exposure of the cores to ultraviolet irradiation. This was determined by densitometry of appropriately exposed fluorograms such as that shown in Fig. 2, and is expressed as a percentage of the radioactivity obtained with non-irradiated cores. Molecular weights of these proteins: ■, 116,000; △, 63,000; ●, 36,500; □, 29,000; ▽, 17,000. *b*, Plot of 17 first-order rate constants, determined from plots such as those in *a*, against the molecular weights of the corresponding proteins. Solid symbols refer to the five proteins shown in *a*. These figures were compiled from data obtained in two independent experiments.

individual mRNAs is made difficult by their poor resolution on gradients and gels, due to a combination of their similar sizes and their heterogeneous poly(A) tails.

Vaccinia cores were exposed to a germicidal ultraviolet lamp for periods ranging from 5 s to 8 min, and then added to the cell-free system. After incubation for 1 h, fresh lysate was added, and incubation continued for a further 1 h. In these conditions the mRNA produced does not saturate the system, so that competition between mRNAs is avoided. The protein products were analysed on SDS-polyacrylamide gels, and their synthesis measured either by densitometry of appropriately exposed fluorograms²⁰, or by direct counting of excised protein bands. The results are shown in Figs 2 and 3. The decay of the synthesis of each protein obeyed single-hit kinetics, but the rate constant varied according to the protein (Fig. 3*a*). Fig. 3*b* plots the rate of inactivation of the synthesis of the 17 most prominent proteins against their molecular weights, which were estimated relative to a set of marker proteins run on the same gel. Thirteen of these bands, including the largest at 116,000, comigrate on SDS gels with proteins labelled in infected cells; at least four correspond to major polypeptides which comigrate on two-dimensional gels with authentic vaccinia-induced proteins (H. R. B. Pelham, unpublished). The other bands are probably not the result of premature termination of translation; analysis of partial digests of the products after labelling with formyl-³⁵S-methionine indicates that they do not share common N termini (H. R. B. Pelham, unpublished).

There is a linear relationship between the molecular weight of the proteins made *in vitro* and the rate of ultraviolet inactivation of their synthesis (Fig. 3*b*), which argues strongly against tandem polycistronic transcription of their mRNAs. If such transcription were happening, there would be proteins whose synthesis was much more sensitive to ultraviolet light than would be expected from their size. This experiment does not exclude the possibility that the mRNAs are derived from longer monocistronic precursors whose size is directly proportional to the size of the coding regions. This seems intrinsically unlikely, and we have failed to detect any large core-associated precursors to mRNA by pulse-labelling experiments. Such experiments would not, however, detect precursors which were cleaved while still nascent. An argument in favour of the idea that the mRNAs synthesised by vaccinia cores represent primary transcripts has been made by Ball and White¹⁵. Unlike VSV,

vaccinia cores seem to be unable to add caps to the ends of RNA molecules generated by cleavage. Since, as I shall show elsewhere, vaccinia mRNA has a strong requirement for capped 5' ends for its translation, it is difficult to accept that messages produced by a cleavage pathway could be active.

These arguments and the data presented above strongly suggest that the major mRNA species produced by vaccinia cores are uncleaved transcripts of single cistrons, modified by the addition of poly(A) at the 3' end and a cap at the 5' end.

The coupled system used in this work is the first example of a eukaryotic cell-free system in which efficient RNA and protein synthesis are directed by a DNA template. It thus offers an *in vitro* analogue of infected cells, and should be useful for studying further aspects of gene expression in vaccinia virus.

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Foreign DNA of bacterial plasmid origin is transcribed in crown gall tumours

Agrobacterium tumefaciens incites cancerous growths in dicotyledonous plants called crown gall tumours. Large plasmids in oncogenic *Agrobacterium* strains¹ carry genetic information which is essential for tumour induction^{2,3}. We recently reported that axenic crown gall tumour tissue contains multiple copies of a small part of the oncogenic (Ti) plasmid of the inciting bacterial strain⁴. The mechanism of induction of crown gall tumours thus seems to resemble that of some virally induced animal tumours: the eukaryotic cell is transformed by addition of new genetic information which is stably maintained through subsequent cell divisions⁵⁻⁷. The means by which foreign DNA brings about the tumorous growth pattern of crown gall cells is unknown. The first step in elucidating its mode of action is reported here: we have detected RNA tran-

were resolved into 21 bands by Agarose gel electrophoresis⁴. Plasmid DNA fragments were alkali-denatured in the gel, neutralised and transferred directly to a sheet of cellulose nitrate^{8,9}. The pattern of bands obtained by electrophoresis was thereby reproduced on the sheet, which was then cut into longitudinal strips for separate hybridisation experiments.

Strips were incubated with labelled RNA isolated from clone E9 of a tobacco tumour incited by strain B6-806, and from normal tobacco callus tissue. In addition, a strip was incubated with ³²P-cRNA synthesised⁹ from the whole Ti plasmid genome by *Escherichia coli* RNA polymerase. Autoradiograms of the hybridised filter strips are shown in Fig. 1 together with a photograph of the bands of DNA in the gel used to prepare the cellulose nitrate transfers. Labelled tumour RNA hybridised to a single band of the *Sma* I digest, identified as band 3 by comparison with the photograph of the gel and the autoradiogram of the cRNA hybrid strip. We have shown that this tumour clone contains about 18 copies, per diploid tobacco cell DNA equiv-

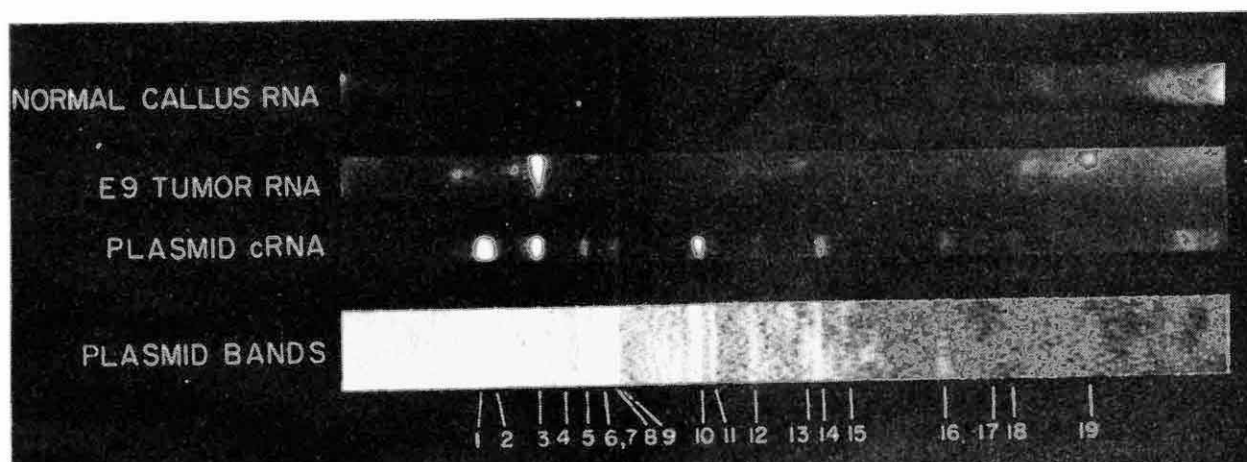


Fig. 1 Hybridisation of labelled E9 tumour and control RNAs to strips bearing *Sma* I digest bands. Tumour and normal callus tissues, tested for sterility at the time of labelling, were incubated in Petri dishes with approximately 1 mCi ³²P (neutralised) per g of tissue for 4 h. RNA was isolated by hot phenol extraction, DEAE-cellulose chromatography, DNase digestion and gel filtration on Sephadex G-75 (ref. 12). Specific activities of RNAs were 127,000 c.p.m. per μ g (tumour) and 34,000 c.p.m. per μ g (normal callus). For cRNA synthesis ³²P-UTP (ICN, 75 Ci mMol⁻¹) was used as labelled precursor, and plasmid from strain A277 served as template. Pure virulence plasmid of *A. tumefaciens* B6-806 was isolated from exconjugant strain A277 and digested with *Sma* I as described previously⁴. After electrophoretic separation in 0.5% Agarose, the bands were stained and photographed⁴ before denaturation and transfer of bands to cellulose nitrate sheets (Schleicher and Schuell, B6 membrane). Strips were soaked in Denhardt's¹³ pre-incubation mixture and baked at 55 °C overnight before use. Hybridisation was carried out with 5×10^6 c.p.m. ml⁻¹ labelled RNA and 200 μ g of unlabelled carrier RNA in 0.3 M NaCl, 0.03 M tri-sodium citrate ($2 \times$ SSC) at 67 °C for 20 h. Filter strips were wetted with $2 \times$ SSC before addition of labelled RNA. Unlabelled carrier RNA was isolated from strain A114, an avirulent plasmidless derivative of strain C58 (ref. 3). After incubation, strips were washed ($2 \times$ SSC 67 °C), treated with RNase (20 μ g ml⁻¹, 1 h, 22 °C), rinsed, dried and autoradiographed for 36 h to 2 weeks. All radioactive label was entirely acid precipitable at the end of the incubation. Autoradiograms of filter strips hybridised with E9 tumour RNA, normal callus RNA and cRNA are presented beside a photograph of the original gel, adjusted to the same scale photographically.

scripts of the foreign genetic information in the tumour cell.

³²P-pulse-labelled RNA was isolated from tumour and normal tobacco callus tissues as described in Fig. 1. To test these RNA preparations for the presence of RNA transcripts of Ti plasmid DNA, we used a filter hybridisation technique developed by Southern⁸. Ti plasmid DNA from the inciting bacterial strain was first cleaved into 25 specific fragments with the restriction endonuclease *Sma* I. These

alent, of part of *Sma* I digest band 3, by direct DNA-DNA hybridisation technique⁴. Our findings show that at least a part of this genetic information is transcribed in the tumour cell. Recent studies have shown that this tumour clone also contains a part of *Sma* I digest band 10 (ref. 10). In three separate experiments using different lots of pulse-labelled tumour RNA from this clone, no hybridisation was noted to band 10 or any other *Sma* I digest bands; only band 3 transcripts were detected. Normal

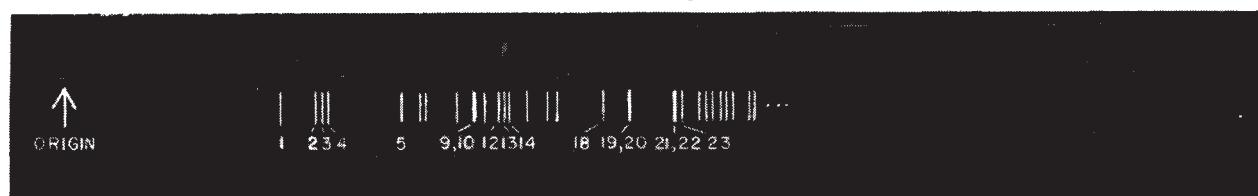


Fig. 2 Hybridisation of labelled E9 tumour RNA to strips bearing plasmid *Eco* RI digest bands. The labelled E9 tumour RNA (5×10^6 c.p.m. ml⁻¹) was incubated for 16 h (67 °C, $2 \times$ SSC) with cellulose nitrate strips bearing *A. tumefaciens* strain A277 plasmid *Eco* RI/(Miles) digest bands (prepared as for Fig. 1). A line drawing of the bands seen in a photograph of the gel is shown below the autoradiogram of the hybridised strip. Hybridisation is assigned tentatively to bands 12, and 21,22. (Close neighbouring bands make this assignment equivocal.) The 6,7 doublet has been proposed to bear virulence functions¹¹.



Fig. 3 Hybridisation of labelled E1 tumour RNA to strips bearing plasmid *Hind*III digest bands. Labelled E1 tumour RNA (3 h pulse, 46,000 c.p.m. per μ g) was isolated as described in Fig. 1. Cellulose nitrate strips bearing *Hind*III (New England Biolabs) digest bands of strain A277 plasmid were prepared, and labelled RNA (4×10^6 c.p.m. ml^{-1}) was hybridised with the strips for 16 h ($2 \times \text{SSC}$, 67°C), washed and RNase treated as for Fig. 1. A line drawing of the bands, prepared from a photograph of the gel, is shown below the autoradiogram of the strip. Labelled E1 tumour RNA hybridisation is assigned to *Hind*III bands 1 and 5.

callus tissue RNA was never observed to bind to any fragment of the plasmid, even after fivefold longer exposure of the autoradiograms.

Further experiments were carried out using ^{32}P -RNA from clone E9 and filter strips carrying bands of *Eco* RI digest of the Ti plasmid. The numerous fragments in this digest (Fig. 2) are not well separated by electrophoresis; thus the identification of bands is imprecise. Tumour RNA hybridised well to a band in the region of band 12, and weakly to a band in the region of the band 21,22 doublet. The latter band, while clearly visible in the autoradiogram, is too faint for photographic reproduction in Fig. 2. Doublet *Eco* RI digest band 6,7 has been proposed to be important in tumour initiation because it seems to be common to distantly related virulence plasmids¹¹. We detected no transcript of this band in this tumour clone. Labelled RNA from normal callus tissue did not bind to any bands on filter strips bearing *Eco* RI digest bands (data not shown).

Labelled RNA from tumour clone E1, a single-cell isolate from the same tumour as E9, hybridised to *Sma* I digest band 3 (data not shown). When this RNA was hybridised to cellulose nitrate strips bearing *Hind*III digest fragments of the Ti plasmid, bands 1 and 5 gave positive results (Fig. 3). Thus these singlet bands appear to contain the same genetic information as the relevant member of the *Sma* I band 3 doublet, which we have identified as band 3b as described elsewhere⁴.

Results presented here show that pulse-labelled crown gall tumour RNA can be used to identify DNA fragments, within any restriction endonuclease digest, which contain Ti plasmid DNA base sequences transferred to the tumour cell. Although not all the foreign DNA is transcribed at detectable levels in the tumour, at least part of the transferred DNA can be detected in this manner. DNA fragments shown to be contiguous by conventional mapping procedures may then be tested for homology with tumour DNA.

Our data confirm, by an independent experimental approach, the presence of a specific part of the Ti plasmid in crown gall tumour cells. Also, these data constitute the first convincing evidence that DNA of bacterial origin is transcribed in demonstrably axenic plant tissue. The possibility must be considered, however, that the transcribed genes in question may ultimately be of plant origin, having been picked up by the *A. tumefaciens* Ti plasmid in the course of its evolution. Should this be the case, one might expect these genes to remain untranscribed in the bacterium. Experiments are in progress to determine whether this is in fact the case. If the same RNA transcripts prove to be present in both the bacterial and the crown gall tumour cell, an unsuspected degree of compatibility between transcriptional processes in eukaryotic and prokaryotic systems will be revealed.

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Decreased initiation factor activity in mouse L cells treated with interferon

INHIBITION of virus-directed protein synthesising events in extracts of cells treated with interferon has been reported^{1–3}. Inhibition of translation of viral mRNAs has been associated with a protein bound to ribosomes of cells treated with interferon^{4,5}. This inhibition may involve the initiation or elongation steps in protein synthesis^{6–7}. Recently, initiation factors for protein synthesis have been isolated from a variety of eukaryotic cells, and it has been established that the initiation factors interact with non-formylated methionyl initiator RNA (Met-tRNA_i) and GTP to form a ternary complex^{8,9}. The experiments presented here show that the activity of initiation factor(s) to form a ternary complex may be impaired or altered in mouse L cells treated with homologous interferon. In preliminary experiments with the initiation factor preparation it was confirmed that the formation of a ternary complex (Met-tRNA_i -eIF-GTP) required GTP for maximal formation (within 2 min at 37°C) and the amount of complex formed was directly proportional to the concentration of initiation factor (Fig. 1). ATP did not substitute for GTP and Mg^{2+} inhibited its formation (data not shown).

When mouse L cells were exposed to (homologous) mouse interferon (100 U ml^{-1}) for 24 h, the initiation factor activity was inhibited about 60% as compared with untreated controls (Fig. 2). A similar decrease in the activity followed a 6-h treatment of the cells with the same unitage of partially purified mouse interferon (specific activity: 10^7 U mg^{-1} protein). As little as 20 U ml^{-1} mouse interferon resulted in significant inhibition (about 18%). It should be noted that the initiation factor activity of interferon-treated cells reached a plateau at a concentration of $90 \mu\text{g}$ protein and the activity gradually decreased at concentrations higher than

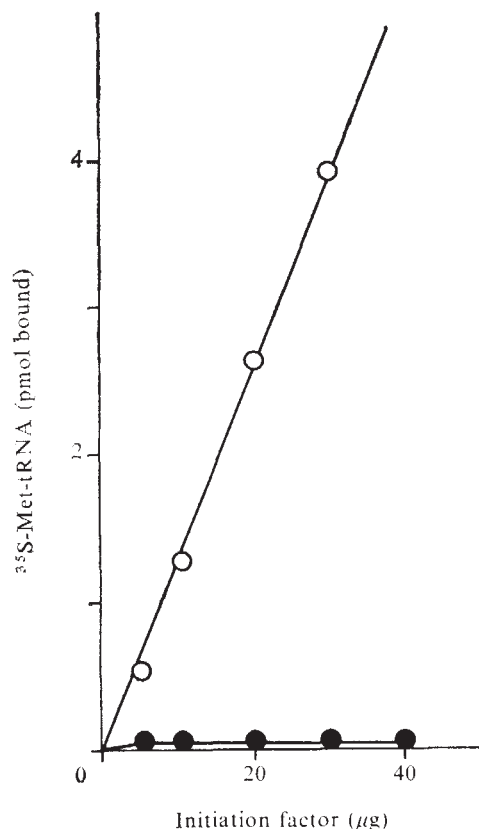


Fig. 1 GTP requirement for formation of ternary complex by initiation factor from mouse L cells. Initiation factor preparation was extracted from exponentially growing mouse L cells treated or untreated with interferon (about 2×10^9 cells). The cells were homogenised in 25 ml 10 mM Tris-HCl buffer (pH 7.5) containing 10 mM KCl, 5 mM $MgCl_2$ and 2 mM dithiothreitol, and centrifuged for 20 min at 12,000 r.p.m. Ribosomes from this supernatant were prepared according to the method of Levin *et al.*⁸. The ribosomes were gently stirred in 8 ml 10 mM Tris-HCl (pH 7.5) containing 0.25 M sucrose, 1 mM EDTA, 2 mM dithiothreitol and 0.5 M KCl for 60 min at 4 °C. After centrifugation at 105,000g for 3 h, the supernatant was precipitated with solid ammonium sulphate (0.361 g ml⁻¹), then re-dissolved in 1.0 ml 10 mM Tris-HCl (pH 7.5), containing 0.1 M KCl, 2 mM dithiothreitol and 5% glycerol, and dialysed against 300 ml of the same buffer for 4 h. All procedures were carried out at 0–4 °C. Rat liver Met-tRNA_f was purified from rat liver using DEAE cellulose¹⁰ and BD-cellulose¹¹ chromatography successively and then charged with ³⁵S-methionine (22.3 Ci mmol⁻¹) using Met-tRNA synthetase purified from *Escherichia coli*⁸. Initiation factor activity was assayed as follows: each 0.1 ml of the reaction mixture contained 40 mM Tris-HCl (pH 7.5), 5 mM dithiothreitol, 0.1 M KCl, 50 pmol ³⁵S-Met-tRNA_f (3,500 c.p.m. pmol⁻¹) and the amount of initiation factor indicated in the text. The solution was mixed on a vortex mixer before and after the addition of initiation factor, then incubated at 37 °C for 5 min in the presence or absence of 5 mM GTP. The complex formation (³⁵S-Met-tRNA_f·eIF·GTP) was determined by dilution with 1.5 ml 20 mM Tris-HCl (pH 7.5), containing 50 mM KCl, 10 mM $MgCl_2$ and 1 mM D-thionine and passage through a nitrocellulose membrane filter (Millipore, type HA). The filter was washed with four additional 2.0-ml aliquots of buffer. After drying, the radioactivity associated with the filter was measured. Protein was determined by the methods of Bücher¹² and Groves *et al.*¹³. The initiation factor from the ribosomes of mouse L cells was prepared and assayed in the presence (○) or absence (●) of GTP. The initiation factor preparation contains initiation factor(s) and ribosomal components which elute from ribosomes and purify in the present conditions.

130 mg (Fig. 2). No effect was detected when mouse L cells were treated with 100 U ml⁻¹ heterologous human leukocyte interferon for 24 h. The reduction in initiation factor activity induced by interferon was prevented by inhibition of cellular RNA synthesis with actinomycin D (98% inhibition of cellular RNA synthesis) or by inhibition of cellular protein synthesis with cycloheximide (93% inhibition of cellular protein synthesis) (Fig. 3a and b). Furthermore, the activity of initiation factor was not inhibited by mouse

interferon added directly to the assay reaction mixture. This finding is not consistent with the proposal that interferon itself is a modified initiation factor¹⁴.

Evidence indicating that the interferon in the preparation was responsible for the altered initiation factor activity comes from the above findings that: (a) partially purified interferon (which originated from a different mouse cell source) had the same effect as crude interferon; (b) heterologous human interferon at levels which did not induce antiviral activity in mouse cells had no effect on the activity of initiation factor; (c) inhibition of cellular RNA synthesis by actinomycin D prevents both the antiviral activity and the alteration of initiation factor activity by interferon; (d) inhibition of protein synthesis by cycloheximide blocks both the antiviral activity and the change of initiation factor activity; and (e) interferon which does not directly inhibit virus, when added directly to the initiation factor assay system did not inhibit the activity (data not shown). Taken together, these findings show that the initiation factor activity from interferon treated mouse L cells was consistently and significantly reduced when compared with the initiation factor activity obtained from control cells. These characteristics coincide with those which are considered to be specific for interferon¹⁵.

A preliminary attempt to compare the physical properties of the initiation factor extracted from control and from interferon-treated cells was carried out by studying their heat stabilities. Initiation factor from cells treated and untreated with interferon were incubated at 50 °C for the times indicated in Fig. 4, and then

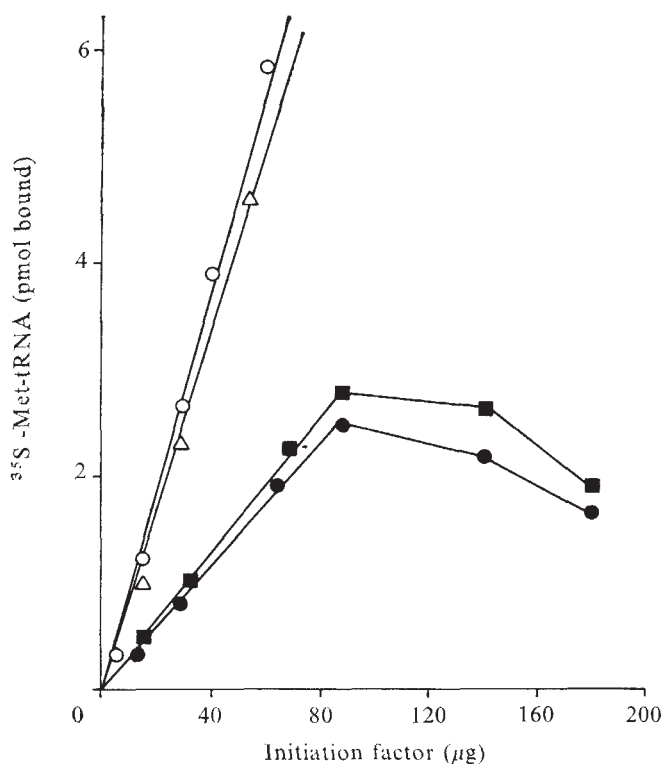


Fig. 2 Comparison of initiation factor activity from mouse L cells treated and untreated with interferon. Exponentially growing monolayers of mouse L cells were treated with 100 U ml⁻¹ of either mouse interferon (Bionetics Corporation) or human leukocyte interferon for the indicated periods in the presence of 2% foetal calf thymus. After treatment of the cells with interferon, they were collected and washed with 10 mM Tris-HCl (pH 7.5) containing 10 mM KCl, 5 mM $MgCl_2$ and 2 mM dithiothreitol. The initiation factors from the cells were prepared and assayed as described in Fig. 1. Initiation factor activity of the cells exposed to mouse interferon (●), human interferon (Δ), or partially purified mouse interferon 100 U ml⁻¹ for 6 h (■), ○, untreated cells. The difference between the average values of initiation factor activity from interferon-treated and control cells observed in eight different experiments are statistically significant ($P < 0.001$ using Student's *t* test).

assayed for residual activity. With a little as 15 min of heating, the initiation factor from interferon treated cells showed greater stability than initiation factor from untreated cells or from cells treated with both actinomycin D and interferon. This finding suggests that interferon either induces an alteration of the normal initiation factor or it induces an inhibitory material which combines with and alters the normal factor. The latter possibility is consistent with the finding that increasing concentration of initiation factor from interferon treated cells results in increasing inhibition of activity (Fig. 2).

Several previous reports have indicated that the translation of added viral mRNAs is impaired in a mixture of extracts from interferon-treated cells more than that of cellular mRNAs³⁻⁵. This inhibitory effect is thought to be mediated by the induction with interferon of a new, cellular antiviral protein which may act by one or more mechanisms: impairment of the binding of viral mRNAs to ribosomes¹⁶; alteration of the methylation of mRNAs¹⁷; restriction of the availability of a minor species of tRNA required for translation^{18,19}; and induction of a nuclease which impairs translation²⁰. The present finding that the initiation factor from interferon-treated cells has diminished activity to form a ternary complex narrows the possible mechanisms in favour of an effect on initiation and will be used in our further studies to help define the basis of selective inhibition of viral translation in interferon-treated cells. Note that the effect of interferon on activity of initiation factor is the earliest event in the sequence of protein synthesis which has been shown to be inhibited by interferon. Other important questions which merit further study include: (a) Can the impairment of initiation factor activity be observed in intact cells treated with interferon? (b) Is the degree of inhibition of initiation factor activity in cells treated with interferon sufficient to account for the reported impairment of the functions of resting, rapidly metabolising and tumour cells? (c) Can the decreased initiation factor activity be overcome by formylated initiator tRNA as has been described for encephalomyocarditis virus polypeptide chains⁶? The latter mechanism may provide a

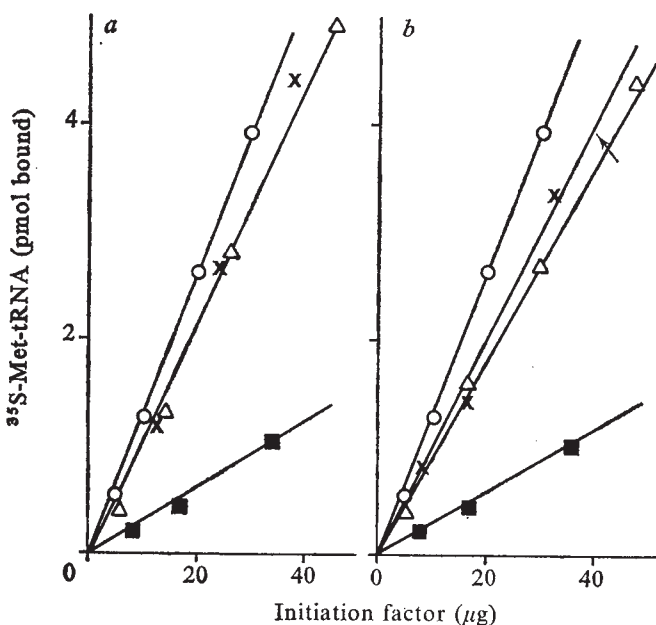


Fig. 3 Effect of actinomycin D and cycloheximide on the impairment of initiation factor activity by interferon. *a*, Activity of initiation factor preparation from L cells exposed to partially purified mouse interferon 100 U ml⁻¹ for 6 h (●), pretreated with actinomycin D 2 μg ml⁻¹ for 60 min, or incubated for 6 h in the absence (○) or presence (Δ) of interferon. Untreated cells (○). *b*, Activity of initiation factor from L cells exposed to partially purified mouse interferon 100 U ml⁻¹ for 6 h (●), pretreated with cycloheximide 100 μg ml⁻¹ for 30 min, or incubated for 6 h in the presence of interferon and cycloheximide (Δ) or in the presence of cycloheximide (○). Untreated cells (○).

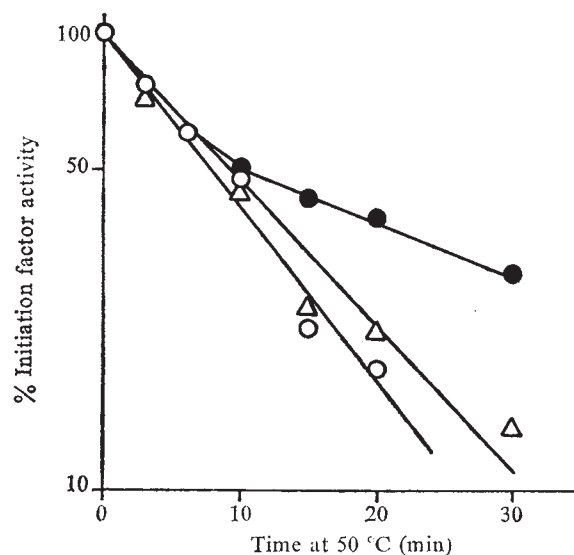


Fig. 4 Temperature sensitivity of initiation factor activity from cells treated and untreated with interferon. Each initiation factor preparation (0.5 mg protein) was incubated at 50 °C for the indicated periods. After heating the initiation factor preparation was tested for ability to form a ternary complex. Initiation factor activities from cells treated for 6 h with partially purified mouse interferon 100 U ml⁻¹ (●), and from interferon-treated cells after treatment with actinomycin D 2 μg ml⁻¹ for 60 min (Δ). ○, Untreated cells.

discrimination between viral mRNAs and host mRNAs at initiation level, because the ternary complex formation in eukaryotic system requires Met-tRNA_i as described previously^{8,9}.

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reviews

Nuclear theory

D. F. Jackson

A Unified Theory of the Nucleus. By K. Wildermuth and Y. C. Tang. Pp. x+389. (Academic: London and New York, 1977.) \$38.50; £27.35.

In nuclear theory the essential problem is to reduce the description of a many-particle system to manageable proportions without the introduction of too many arbitrary assumptions and with the possibility of estimating the effects of approximations made. In the unified theory of nuclear reactions, particularly associated with the name of Feshbach, projection operators are used to reduce the true many-particle Hamiltonian to effective Hamiltonians, which belong to subspaces of the total Hilbert space but contain the correct asymptotic boundary conditions for the final states. In contrast, Wildermuth and Tang propose a theory in which the total wavefunction for the many-particle system is represented as a sum of non-orthogonal terms each corresponding to a final system with the correct boundary conditions. Thus, the subspaces of the total Hilbert space are defined through the wavefunction and, it is argued, this provides the correct asymptotic behaviour for the initial state and all final states.

The authors apply their approach to a variety of bound-state and reaction problems. The terms of the wavefunction each correspond to a possible cluster structure of the many-particle system, although, as the authors repeatedly stress, it is inappropriate to take these cluster structures too literally because of the effect of the anti-symmetrisation operator which exchanges particles between the clusters. Among the problems discussed are the construction of bound-state wavefunctions and the evaluation of charge density distributions, ground-state energies and transition probabilities, the scattering of light systems at low and medium energies, resonance reactions, the microscopic formulation of optical potentials, and fission.

This book covers a very wide range of nuclear physics and contains some penetrating observations about aspects of the theory. It is, however, a rather frustrating book to read. So many topics are started and reach a premature conclusion with a rather trivial

example based on a very light system such as ^8Be or ^7Li . There is a useful discussion of the generator coordinate technique but recent developments in the resonating group method and the orthogonality condition model are ignored. The discussion of optical potentials for composite particles completely ignores extensive work done with folding models. The most serious weakness of the approach is that, although great emphasis is laid on the

need for correct asymptotic behaviour of wavefunctions, all the examples fall back on oscillator functions which are notoriously incorrect at large separation distances. Until the practical application of the theory can be extended to yield the correct asymptotic behaviour the formal theory is unlikely to lead to major advance. □

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Cancer chemotherapy

Biosynthetic Products for Cancer Chemotherapy. Vol. 1. By G. R. Pettit. Pp. xii+215. (Plenum: New York and London, 1977.) \$23.40.

The author states that his main aim is "to provide a current overall view of the cancer problem and the development of cancer chemotherapeutic drugs of biosynthetic origin". If you want a reasonably detailed summary of the chemical and biological properties of the kinds of cytotoxic products which have been isolated from plants, micro-organisms, marine organisms, reptiles and other sources, then this book, from p47 on, may supply your needs.

It is less easy to know for whom the whole book was written. The first chapter (Introduction and Perspective) is the longest (46 pages); in it, the author discusses the nature, causation, and treatment of cancer with special emphasis on the role of the National Cancer Institute. Such a survey seems out of place and belies the title of the book. Dr Pettit could undoubtedly write a useful review or two on different aspects of cancer chemotherapy, but not, as here, by trying to cram a quart into an inappropriate pint pot.

In his introductory chapter, the author refers to the ten or so malignant conditions particularly amenable to drug treatment, without making it clear that these are relatively rare cancers. Their control is a commendable achievement, but is only a scratch on the surface of the cancer problem.

On the credit side, the author and publishers are to be complimented on the clear and numerous structural formulae. They occupy, however, all

of 80 pages, leaving only about 75 pages of text for the natural products section of the book. Dr Pettit has succeeded in compressing a large amount of information into these pages, and the comprehensive tabular survey which volume 2 will provide, promises to be a useful supplement.

The author's enthusiasm often comes through, insufficiently tempered, perhaps, by an acknowledgement of the limitations of cancer chemotherapy in man at the present time. He clearly feels that much more money could usefully be spent on the exploration of natural products as potential anti-tumour agents. He may be right, but some of his statements would not find universal acceptance. Take, for example, the dogmatic comment on p175: "Given sufficient financial support and time, the lower animals will prove to be a particularly good source of unusual and valuable drugs for cancer chemotherapy". Again, the author seems to imply (p58) that, with sufficient knowledge, one could design a single substance effective against human cancer in its various forms, a view now held by few.

Such criticism does not invalidate the work as a handy reference book on a range of naturally occurring cytotoxic compounds, although—if the reviewer may now be dogmatic—the very great majority of these products will never become established as clinically useful drugs. One must therefore not take the title of the book too literally.

J. A. Stock

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Inorganic azides

Energetic Materials. Vol. 1: Physics and Chemistry of the Inorganic Azides. Pp. 503. \$59.40. Vol. 2: Technology of the Inorganic Azides. Pp. 296. \$90. Edited by H. D. Fair and R. F. Walker (Plenum: New York and London, 1977).

THESE volumes review recent research and development work in the physics, chemistry, and technology of the inorganic azides. Summarising fundamental advances over the past two decades, emphasis is placed on investigations supported by the United States Army through its Office of the Chief of Research and Development and its Materiel Command. The contributing authors are, for the most part, members of the Energetic Materials Division (formerly Picatinny Arsenal), Armament Research and Development Command, New Jersey.

The wide variation in crystal bonding characteristics and stability of the inorganic azides present problems of unique interest in solid state physics and chemistry. In the reactive state, the inorganic azides exhibit a remarkable range of reactivities from low order decomposition, deflagration, to high order detonation. These properties have led to the commercial development of azides in a wide field, including particularly gas-generating devices and explosives, as well as photographic emulsions.

One of the central and fundamental problems in the study of azides lies in the development of valid equations of state applicable to the explosive decomposition of these materials. This requires the elucidation of interatomic forces from data on vibrational levels obtained from crystal spectra. Considerable success along these lines obtained with the solid alkali metal halides, using dynamic models, has very recently been applied to the inorganic azides, using laser Raman and thermal neutron spectroscopy. The results of these studies have contributed greatly to our understanding of lattice and phase transition dynamics in the crystalline metal azides. Another important area concerns the calculation of activation energies of decomposition, and the correlation of these with ground and excited energy levels in the azide crystal.

These major aspects are reviewed comprehensively in Volume 1, which deals with azides across the whole range of the Periodic Table, with particular reference to azide crystals and crystal structure; molecular vibrations in lattices; electronic structure of the

solid state; and all aspects of decomposition, initiation and propagation in solid metal azides.

Volume 2 is devoted to the technology of those metal azides used in detonators, and as such is largely concerned with lead and silver azides. The aspects covered comprise manufacturing techniques and process control; analysis of azides; handling and safety; sensitiveness and the roles of azides in detonating trains. The section on electrostatic sensitivity and dielectric properties of lead azide and other primary explosives is of great current interest. The final section on the initiation of detonating trains by azides, also merits comment. In these situations lead azide functions commonly both as

acceptor and as donor. These two processes together determine the pulse time and shape of the impulse integral on which the detonation threshold of the secondary charge depends. Recent high precision data are presented on these phenomena.

In conclusion, these volumes offer an extremely comprehensive, well-documented review of the most significant aspects of the science and technology of the inorganic azides. This work will be of inestimable value, particularly to those concerned with the development of military and commercial explosives. **J. H. Turnbull**

J. H. Turnbull is Professor and Head of the Chemistry Branch, Royal Military College of Science, Shrivenham, UK.

Aerosol research and technology

Smoke, Dust and Haze: Fundamentals of Aerosol Behaviour. By S. K. Friedlander. Pp. xvii+317. (Wiley: New York and London, 1977.) \$19.50; £11.35.

AEROSOL research and technology is, like most modern fields of interest, complex and interdisciplinary. Whereas transport, deposition, collision and coagulation are merely governed by physical laws, the formation of particles is largely a chemical problem. Meteorological processes such as rain-out, wash-out, and boundary layer physics are involved as well as engineering aspects for the design of sampling and measuring devices.

The available literature is specialised on certain aspects of the complex field, and there is a strong need for a comprehensive presentation especially for those who want to get into this field. Friedlander's book is likely to close a great deal of this gap. Written as a classroom text for graduate students in environmental engineering it mainly covers the physical and engineering aspects of aerosol behaviour.

The first third of the book is devoted to particle transport and deposition. Excellent fundamentals of problems, such as diffusion and deposition for different flow parameters, filter efficiencies, electrical precipitation, inertial and turbulent deposition, are given.

The second third deals with fundamentals of particle generation and growth. Collision and coagulation processes, thermodynamic processes, such as condensation, formation and stability of droplets and clusters, and a brief outline on gas-to-particle conversions, are presented.

In the last part, a general dynamic equation for the continuous distribution function is set up taking into account all processes of particle formation, growth, diffusion, coagulation, convection, and sedimentation discussed before. Special cases, in particular the dynamics of turbulent stack plumes, are discussed and compared with observational data. The techniques for setting up predictive models for air quality-emission source relationship are briefly described.

A short chapter on optical properties is confined to light scattering and visibility. For the complete problem of radiative transfer the reader is referred to the specialist literature. In another short chapter on experimental methods, descriptions of sampling, filtration, particle counters, the cascade impactor, devices for chemical analysis, and aerosol generators, are given.

The book is clearly written and beautifully made. At the end of every chapter carefully selected examples and problems provide excellent applications of the fundamentals treated. Friedlander concentrates mainly on the physical aspects of aerosol behaviour. Important chemical processes such as gas-to-particle conversion through heterogeneous reactions and meteorological aspects of particle transport and precipitation are only briefly outlined for completeness. It would be highly desirable to see equally well produced books covering the chemical and meteorological aspects being published.

This book is more than a textbook for graduate students in environmental engineering. It should be on the bookshelf of every scientist and engineer directly or indirectly involved in aerosol research and technology. **P. Fabian**

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Molecular weight determination

Molar Mass Measurements in Polymer Science. By N. C. Billingham. Pp.254. (Kogan Page: London, 1977.) £13.50.

DISCUSSION of the theories underlying molecular weight determinations is almost an incidental part of most textbooks of polymer science. In contrast, practical aspects of the various techniques are most commonly discussed in the instruction manuals issued by appropriate manufacturers. The author of this book has made a highly successful attempt to combine the two sources of information into a most useful and reasonably concise reference text.

As the title indicates, the book is written with a fairly rigid adherence to the SI system of units; molar mass, a fundamental unit with dimensions, being preferred to the more generally used, dimensionless quantity, molecular weight.

In the early part of the book the various molar mass averages, and their relationships, are discussed as a preliminary to a most enlightening survey of the nature and behaviour of polymer molecules in solution. Membrane

osmometry, vapour pressure, light scattering, ultracentrifuge, viscosity, and gel permeation chromatography techniques are fully described in later chapters and, for each, there is a discussion of both underlying theory and practical laboratory procedures. Different types of instruments supplied by various manufacturers are individually described and compared, and full details of equipment suppliers and a list of suitable solvents for most common polymers are included as appendices. There is also a very short discussion of chemical methods for assay of endgroups in polymers.

In total, the contents of this book are rather similar to those of two edited volumes (*Polymer Molecular Weights*, ed. P. E. Slade, Jr, recently published) and indicate the requirement for a convenient reference text of this kind. The present single volume, however, has the advantages of both lower price and a more coordinated treatment of the subject. It will be valuable to anyone interested in polymer characterisation and especially to research workers who may be abruptly faced with the problems of molecular weight determination.

A. Ledwith

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Liquid-state chemistry

Introduction to Liquid State Chemistry. By Y. Marcus. Pp. viii+357. (Wiley-Interscience: London and New York, 1977.) £12.50; \$24.00.

LIQUID-STATE PHYSICS is a flourishing branch of science which has been reviewed recently in several good books. The liquids discussed were those whose structure is the most easily determined, and whose properties provide the most direct test of physical theories; argon is the archetype. Professor Marcus has a different aim—to give an account of the equilibrium properties and structure of those liquids that are of more concern to the chemist. "Crude petroleum, sea water, aqueous acetone and molten cryolite" are his examples.

Before tackling his main subject, he rightly supposes that his reader will need first to know something of the physics of simple liquids and their mixtures, and so the first half of his book is essentially liquid-state physics. Unfortunately, 160 pages is too small a space for this to be anything but a string of theories, models, approximations, and references. Those familiar with the field might wish that he had been more critical in his choice of material; those who are not, will probably gain little but a list of references and useful tables, since the text is not only too condensed but abounds with errors and examples of misplaced emphasis.

The second half of the book covers his main interest; there are chapters on mixtures of molecular liquids, solutions of electrolytes, molten salt mixtures, and mixtures of liquid metals. Here, the level of rigour of the physics is necessarily lower than in the earlier chapters; often a well chosen correlation of physical properties is more valuable than a neat trick with the grand partition function. Chemists who work with liquids will welcome the last four chapters, with their extensive tables and full references.

The units of the book are said to SI, but this system implies not only the use of metres, kilogrammes, seconds, and so on, but also the use of the rationalised electromagnetic system. Unfortunately, the author has married the sizes of the units of the SI with equations of the unrationalised electrostatic system, so that we have, for example, a polarisability in $\text{m}^3 \text{mol}^{-1}$. This combination will probably confuse students reared on the *Système International*, and not only on its units.

J. S. Rowlinson

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Advances in Invertebrate Reproduction

Volume I

Edited by K. G. Adiyodi & Rita G. Adiyodi
1977; xii+514pp.; art paper; hard bound; £23/\$40
(inclusive of postage)

Proceedings of the First Symposium of the International Society for Invertebrate Reproduction, Calicut, September 10-12, 1975. Updated and revised. The 39 original contributions and overviews by leading authorities in the field cover several fascinating aspects of sexuality, reproduction and behaviour of a wide range of invertebrates. Some of the comparative data given on vertebrates add to the usefulness of this volume. Profusely illustrated and complete with author, subject and species indexes.

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Precambrian formations

Precambrian of the Northern Hemisphere. By L. J. Salop. Pp.ix+378. (Elsevier Scientific: Amsterdam, New York and Oxford, 1977.) Dfl.145; \$59.25.

THIS book provides an "inter-regional correlation of Precambrian formations", the subdivisions of which are based on the "natural stages in the earth's evolution". Essentially the book is a correlated list of the Precambrian formations of the northern hemisphere.

Three introductory chapters are largely concerned with methods and principles of correlation and subdivision, and the two final chapters review briefly the major features of geological evolution during the Precambrian (tectonic models and plate tectonics are avoided). The six main chapters list the principal rock groups in Salop's six subdivisions, as follows: (1) The Archaean era includes all rocks formed more than 3500 Myr ago (largely gneisses and granulites). (2) The Paleoprotozoic ("Protozoic" is Salop's new term) (3500–2600 Myr) which concerns the well-known greenstone belts. (3) The Mesoprotozoic (2600–1900 Myr) including geosynclines like Krivoi Rog, the Huronian and the Labrador Trough. (4) The Neoprotozoic (1900–1000 Myr) which deals with clastics and red beds in platforms and aulacogens (for example, Gardar and Keweenaw) and the gabbro-anorthosite-rapakivi granite-alkali syenite complexes. (5) The Epi-protozoic (1000–650 Myr) including platform and miogeosynclinal, mostly terrigenous (especially red bed) facies and the prominent tillites (for example, lower Dalradian, upper Briovarian). (6) The Eocambrian (650–570 Myr), dealing with platform clastics and the Ediacara fauna.

There are about 800 references, at least half of which relate to the USSR and China, but most date from the 1960s. A disappointing aspect of the book is the paucity of figures—only 36 with an additional four tables. Two

very useful charts in a back pocket correlate formations in 78 regions across North America and Greenland, and 78 across north-western Europe and the USSR (Britain is missing); and a large table gives a synopsis of all aspects of geological evolution.

Salop is very much better on the younger than the older Precambrian—in fact, his interpretations of some Archaean formations will raise many eyebrows. All gneissic-granulite areas have to be Archaean and their stratigraphy is sedimentary (V. R. McGregor's tectonic intercalations are not considered). Isochrons are said to indicate "rejuvenated" ages dating the period of late recrystallisation. Thus, the Fiskensæset complex, the Scourian

and the Moldanubian are considered to have "formed" more than 3500 Myr ago, but the Isua supracrustals are included in the Paleoprotozoic. Salop's classification is so rigid that it does not allow greenstone belts to form in one region while high-grade gneisses are forming in another.

The book provides, however, for the first time in English a very valuable correlation of the main formations and rock groups throughout the USSR and China with those "in the west". This is its main contribution.

B. Windley

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Modern knowledge of cyclic nucleotides

Cyclic Nucleotides in the Nervous System. By John Daly. Pp.401. (Plenum: New York and London, 1977.) \$39.

ONE of the first papers from Earl Sutherland's laboratory about the new nucleotide, cyclic AMP, included observations on the distribution in mammalian tissues of adenylate cyclase, the enzyme responsible for its synthesis. The highest concentration was found in the brain. Thus, it was clear from the start that Sutherland's epoch-making discovery would figure prominently in neurobiology. Development of the significance of this observation was initially due to Sutherland's co-worker, T. W. Rall, who observed that in the nervous system cyclic AMP functions as second messenger not to hormones circulating in the blood stream, but to certain neurotransmitters. Rall's work may be said to have set the stage for the tremendous expansion in the literature on cyclic nucleotides in the nervous system so extensively documented in John Daly's monograph.

Dr Daly's book is entirely orientated to the nervous system; no concessions are made to the general reader unfamiliar with the background to the subject. After a cursory introduction of no more than two pages, he launches into the first of three long chapters, entitled "Enzymatic Formation, Degradation and Action of Cyclic Nucleotides". This deals mainly with the pro-

perties and distribution of adenylate and guanylate cyclases and phosphodiesterases in brain, ganglia and cell cultures, together with a description of enzyme systems concerned in the cyclic AMP-dependent phosphorylation and dephosphorylation of tissue proteins. Next comes an extensive account of the *in vitro* accumulation of cyclic nucleotides in cell-containing preparations of brain, ganglia and other cells of neural or glial origin. The detail here is exemplary, reflecting the author's personal research interests. The final chapter deals with "Functional Roles for Cyclic Nucleotides in the Nervous System". Understandably at this stage of our knowledge it concentrates on actions and effects rather than the physiological functions, of which Dr Daly would be the first to recognise very little is known at present.

The book should prove an excellent text for researchers about to enter or already working in the field. It contains over 1,300 references and is written in a straightforward style that expounds facts and eschews speculation. Such an approach does not always make for easy reading, especially since very little effort is made to summarise or synthesise the mass of information, or indeed critically assess its relative importance. This leads to some repetition and the occasional uncritical citation of dubious work. Dr Daly's book is therefore about the 'trees' rather than the 'wood' but is nevertheless invaluable as an exhaustive source of up-to-date information on modern knowledge of cyclic nucleotides in the nervous system.

R. Rodnight

R. Rodnight is Professor of Neurochemistry at the Institute of Psychiatry, London, UK.

Erratum

● In the review of *Spectrochemical Analysis of Pure Substances* (Nature, 11 August, 268, 572, 1977) the publisher was incorrectly quoted. This should have read: Adam Hilger: Bristol; Crane, Russak: New York.

obituary

Lord Adrian, 1889–1977

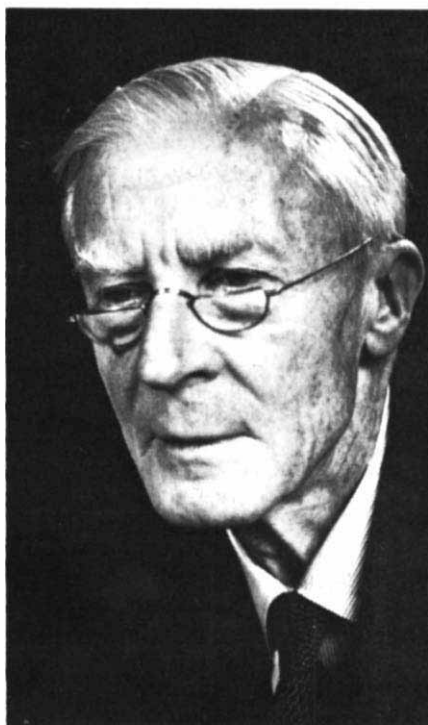
LORD ADRIAN disliked his christian names, Edgar Douglas, and was invariably known to his family and friends as Adrian; he will be called Adrian in this article which is concerned mainly with his scientific work, and particularly with the events which led up to the discoveries described in his first two books *The Basis of Sensation* (1928) and *The Mechanism of Nervous Action* (1932).

In order to understand the full extent of Adrian's contribution to science and medicine we must first consider the changes which have come over physiology since he started work with Keith Lucas in the Physiological Laboratory at Cambridge in 1911. It is easy to appreciate the effect of the development of electronic equipment for, as Adrian himself once said, "The history of electrophysiology has been decided by the history of electric recording instruments". But there have also been overwhelming changes in ideas and these are harder to take in, for, as historians of science know only too well, it is extraordinarily difficult to put ourselves in the position of our scientific ancestors who did not accept some scientific law or principle of nature that we now regard as self-evident.

One must, for example, remember that although Bowditch established the all-or-nothing law for heart muscle in 1871, in 1911 it was still uncertain whether the same law applied to nerve. The work which Adrian did before 1914 helped to show that in a single nerve fibre the size of the impulse is independent of the strength of the stimulus, provided the latter is large enough to evoke a response; it also removed some of the difficulties which had persuaded scientists as perceptive as Max Cremer or F. Hofmann of the opposite.

Then there was a complete muddle about the contraction of skeletal muscle and it was widely held that the quick and slow movements of voluntary muscle were brought about by different contractile mechanisms activated by two types of nerve fibre, or even by two forms of nerve impulse. This situation was not cleared up until Adrian and Bronk carried out their famous experiments in the late 1920s.

Adrian, who was not at all fussy about laboratory accommodation, has



Douglas Glass

given an entertaining description about life in the old Physiological Laboratory at the time when he started work there. "In those days the Cambridge School of Physiology was at the height of its fame. It was housed deplorably, judged by modern standards, and run so economically that there was no mechanic and no common stock of tools or apparatus. The research rooms were barely rooms at all. Keith Lucas was lucky to have a small cellar all to himself. It was approached through a larger cellar which housed A. V. Hill and many cages of rats on which Hopkins was carrying out his classical work on the vitamins. A side door led to a dark chamber in which all the frogs were kept and beyond this was the centrifuge driven by a large gas engine of obsolete design which shook the building and added the smell of warm oil and half-burnt gas to that of frog and rat: upstairs worked Barcroft, Mines, [W. B.] Hardy, Anderson, Fletcher, Langley, to name only those who were in permanent occupation, and their accommodation was little better."

In 1913 Adrian wrote a thesis on *The conduction of the impulse in the nerve fibre* which won him a Fellowship at Trinity College, of which he was later to become Master (1951–1965).

In July 1914 Adrian went to St Bartholomew's Hospital where he qualified in record time, just over a year; he then worked on nerve injuries and shell shock, first at Queen Square and later at the Connaught Military Hospital at Aldershot where he remained to the end of the war in spite of strenuous efforts to get to France.

There are several interesting papers of a clinical nature from this period, but much the most remarkable is one published in 1917 in the *Lancet* with L. R. Yealland on the treatment of some common war neuroses. The paper describes a highly successful method of dealing with hysterical paralysis, which the authors applied with at least 95% success to more than 250 cases, including all the common hysterical disorders such as mutism, deafness, paralysis of limbs and so on. No great originality was claimed for the method which depended on persuasion and suggestion, usually employing electrical stimulation to evoke sensation or movement, but it is evident that the authors applied the method with much ingenuity and understanding of human nature. The article is also remarkable for sympathetic references to psychoanalysis which bear witness to the width of Adrian's interests, as does the manuscript of an unpublished lecture 'Freud without tears' given in 1919.

After the war, Adrian continued for several years with analytical electrophysiology of the type which he had started with Keith Lucas (who was killed in an aeroplane accident in 1916). But about 1925 he began to use valve amplifiers and made what in the jargon of today (which Adrian detested) would be called a break-through.

The transformation is best described in his own words. "Alexander Forbes had been working with me in Cambridge and I had learned a great deal from him about string galvanometers and about mammalian preparations, but the experiments I had started became more and more unprofitable. You know the sort of thing that happens—they became more and more complicated and the evidence more indirect, and after a time it was quite clear that I was getting nowhere at all."

Adrian then describes how he had built a 3-stage valve amplifier to the design given him by Gasser and had

connected it up to a capillary-electrometer recording from the sciatic nerve of a nerve-muscle preparation. He was distressed by a rapid electrical oscillation which appeared whenever the muscle was hanging down freely and disappeared when it was supported. Then "The explanation suddenly dawned on me, and that was a time when I was very pleased indeed. A stretched muscle, a muscle hanging under its own weight, ought, if you come to think of it, to be sending sensory impulses up the nerves coming from the muscle spindles, signalling the stretch on the muscle. When you relax the stretched muscle, when you support it, those impulses ought to cease.

"I don't think it took more than an hour or so to show that that was what the little oscillations were. I was able to make photographic records of them, and within about a week I was nearly certain that many of these oscillations were action potentials coming up sensory fibres in the nerve, and what was more, that many of them came from single nerve fibres and that by some extension of the technique it ought to be possible to find out exactly what was happening in single nerve fibres when the sense organs attached to them were stimulated.

"That particular day's work, I think, had all the elements that one could wish for. The new apparatus seemed to be misbehaving very badly indeed, and I suddenly found that it was behaving so well that it was opening up an entire new range of data. I'd been bogged down in a series of very unprofitable experiments and here suddenly was the prospect of getting direct evidence instead of indirect, and direct evidence about all sorts of problems which I had set aside as outside the range of the techniques that one could use. The other point about it was that, as I said, it didn't involve any particular hard work, or any particular intelligence on my part. It was one of those things which sometimes just happens in a laboratory if you stick apparatus together and see what results you get."

The comment that one wants to make about the last sentence is that when most people stick apparatus together and look around they do not make discoveries of the same importance as those of Adrian.

The initial advance made by Adrian was rapidly followed by three important papers with Zotterman which showed beyond doubt that the nerve impulse was invariant, that the intensity of sensation was conveyed by the frequency of nerve impulses and the quality by the type of nerve fibre in action. Another very important point is that adaptation to a steady stimulus takes place peripherally and that some

sense organs, like those concerned with touch, are rapidly adapting, whereas others like muscle spindles are slowly adapting.

These early experiments on sense organs are beautifully described in *The Basis of Sensation* which is written in such simple and elegant language that it is difficult for a student of today to appreciate the novelty and importance of what is being said.

At about this time Adrian's small group was joined by B. H. C. (now Sir Bryan) Matthews who followed up Adrian and Zotterman's work in two classical papers on muscle spindles. Matthews also introduced the oscilloscope which bears his name and which proved extremely suitable for the type of work done by Adrian and his school. Later Adrian and B. H. C. Matthews collaborated in important studies of the Berger rhythm and on the interpretation of potential waves in the cortex; these papers laid the foundation of modern work on electroencephalography in man.

Other famous collaborative papers from the period 1926–1944 were those with Rachel Matthews on the action of light on the eye, with Bronk on the discharge of impulses in motor nerve fibres and with Moruzzi on the motor cortex and pyramidal tract. The paper with Bronk cleared up the long-standing puzzle of the origin of 'tonus' in mammalian skeletal muscle by showing that during weak discharges motoneurons fire at slow rates, ten per second or so, giving an incomplete tetanus in individual motor units but a smooth movement of the whole muscle because the discharges are not synchronised.

In 1937 Adrian who had previously held a Foulerton Research Professorship of the Royal Society, succeeded Sir Joseph Barcroft as Head of the Department of Physiology, a post which required much teaching and administration and which he held until 1951 when he became Master of Trinity.

In addition, Adrian was President of the Royal Society from 1950–1955, of the British Association in 1954 and of the Royal Society of Medicine from 1960–1961, Chancellor of Leicester University from 1957–1971, Vice-Chancellor of Cambridge from 1957–1959 and Chancellor of Cambridge from 1968–1976.

Adrian shared the Nobel Prize with C. S. Sherrington in 1932, was awarded the Order of Merit in 1942 and received a host of other honours which testify to the esteem in which he was held throughout the world; he was made a peer in 1955. He was a superb lecturer and after-dinner speaker and was much in demand in both capacities;

in addition he served on several time-consuming and important committees such as the Medical Research Council, the University Grants Committee and the Chemical Board.

After contemplating the list of positions held by Adrian from 1937 onwards one might be forgiven for thinking that he had little time for research in the Laboratory, or at least that he would be forced to rely mainly on help from colleagues. This was very far from the truth. From 1937 till 1959 Adrian was constantly in the Laboratory and published a number of excellent papers dealing with subjects as diverse as hearing, the sense of smell, vision, the cortex and the thalamus, and using animals as large as a pig or a Shetland pony or as unusual in a physiological laboratory as an alligator or a hedgehog. Much of this work was done alone, Adrian's custom being to dispense with the help of his faithful assistant Hatton as soon as the animal was anaesthetised and a single pair of hands could cope.

Edgar Douglas Adrian was born on 30 November 1889. He read classics at Westminster School and became interested in philosophy, partly at least through his friendship with C. D. Broad. This interest can be seen in all three of his books but most strikingly in *The physical basis of perception* which skates round the problems of mind, brain and consciousness in a most engaging manner. The book also contains a fascinating summary of the experiments which he carried out during the latter part of his scientific life.

In 1923 Adrian married Hester Pinsent who later became famous for her work on mental health; she died in 1967 to the sorrow of a wide circle of friends. Adrian relied greatly on her enterprise and ability and there is no doubt that she made an important if indirect contribution to his scientific achievements. They had three children, Mrs Anne Keynes, Mrs Jennet Campbell and Richard Hume Adrian, FRS who succeeds to his father's title.

After Hester's death, Adrian returned to Trinity, the College which he loved so well, to live in a beautiful set of rooms in the corner of Neville's Court. Until failing health intervened, it was his custom to entertain scholars and many other undergraduates to lunch or dinner about once a week. He remained in Trinity until a few weeks before his death in the Evelyn Nursing Home at the age of 87 on 4 August 1977.

A. L. Hodgkin

¹ *The Mechanism of Nervous Action*, 2. (Oxford University Press, 1932).

² From Adrian's chapter in the account of Keith Lucas published in 1934 (W. Heffer: Cambridge), 90–91.

³ *Memorable experiences in research* by F. D. Adrian and others. (*Diabetes*, 3, 17–27, 1954).

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LKB Microbiological Flow Calorimeter

Portable flocculator. Voss. This portable flocculator measures $52\text{ cm} \times 33\text{ cm} \times 18\text{ cm}$, is lightweight and can be carried easily. When the case is opened it acts as a base for the flocculator. It consists of a 4-bank stirring unit with flat propeller blades $60\text{ mm} \times 25\text{ mm}$. The diameter of the stirrer shafts is 6.3 mm and the centre between paddles is 10 cm. When paddles are withdrawn there is a clear height from base to underside of paddle of 15 cm. The stirrers are driven by a geared motor at speeds between 25 and 250 r.p.m. A thyristor control enables fine speed control.

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Replica plating set. Embio Equipment.

A labour-saving equipment for the replica plating technique has now been developed which uses glass beads, a picking device, a bead sieve and a new type of replicator. A sterile glass bead is sucked by vacuum on to the tip of the picking device, by covering a hole in the device with a finger. The bead, so held, is dipped into a bacterial colony and immediately transferred to a hole in the sieve, which is in position on a nutrient agar plate. The glass bead is left in the sieve and the procedure is repeated with another glass bead. The bead sieve can accommodate up to 100 beads on a single 9-cm plate. The bacteria-carrying beads are thus inoculating the plate. The beads and the sieve are then easily removed from this plate. Additional replicates of the colonies that appear on this master plate after incubation can be made by using the replicator. Twenty successive replicas are easily obtained.

Circle No. 47 on Reader Enquiry Card.

Digital film thickness monitor. Nanotech. Many vacuum deposition processes require the control and indication of increasing film thickness during the deposition cycle. The model NQC1 is an economical instrument for this purpose. The deposited film thickness is read directly on a 4 digit LED display, a thumbwheel switch allows the density of the material being deposited to be entered to ensure the correct thickness reading using any deposition material. The thickness of the deposited material is measured by the alteration in the resonant frequency of a quartz crystal located close to the material being coated. The maximum thickness that can be measured before replacing the crystal is equivalent to $12\text{ }\mu\text{m}$ of aluminium or proportionately less for more dense materials. Alternative configurations of crystal holder are available to suit different applications. These include water cooled sensors for high temperature ambients, also special filter arrangements to extend the quartz crystal life in high rate deposition processes such as Ion Plating.

Circle No. 48 on Reader Enquiry Card.

Plasma etching unit for electron microscopy. Nanotech. The Plasmaprep 100 includes a Pyrex chamber 150 mm × 100 mm diameter which is externally excited by a variable supply of up to 100 W RF power. Access to the chamber is gained by a vacuum sealed hinged door, the pressure inside the process chamber is controlled by a gas inlet needle valve with associated flow meter. The chamber is evacuated by a rotary vacuum pump to a normal working pressure in the 0.5 to 1.0 torr region. Control of the etching process is simplified as the equipment is semi-automated; when the chamber has been evacuated the RF power can be varied and tuned to match the chamber pressure and loading. The choice of reagent gas dictates the reaction with the specimen; typical gases used are oxygen, hydrogen and fluorinated and chlorinated hydrocarbons. Reaction products are removed in the gas stream.

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Nanotech plasma etching unit

Rotating anode X-ray generator. Marconi-Elliott. The new rotating anode X-ray generator operates at currents up to 300 mA at 50 kV on a focus of 0.5 mm. This new high power (15 mA kW) generator greatly extends the field of application and brings the advantage of short counting and exposure times as well as focal spot sizes down to 0.1 mm to all crystallographers in both research and industrial laboratories. It is the first X-ray diffraction equipment to comply in every way with the forthcoming statutory safety regulations under the Health and Safety Act. The GX21 generator has been specially designed to prevent the occurrence of any accidental exposure of personnel to a significant level of dangerous radiation. A transparent radiation shield can be provided to protect the operator from hazards from all diffraction instruments which are not themselves shielded.

Circle No. 50 on Reader Enquiry Card.

Disposable filter units. Variable Volumetrics. Completely disposable miniature liquid and gas filters, Balston Microfibre Disposable Filter Units are

specially packaged for laboratory use in boxes of five filters each and are available in a range of filtration grades which make them suitable for such laboratory functions as filtration of reagents and samples to liquid or gas analysers, filtration of liquids fed to liquid chromatographic columns, filtration of precious metal solutions without danger of leakage, sterilisation of compressed air and other gases, and complete removal of oil, dirt and water from compressed air and other gases. The units are also ideal for use as vent filters on microbiological reactors to prevent loss or escape of microorganisms.

Circle No. 51 on Reader Enquiry Card.

Industrial conductivity transmitter. Philips. The PW 9521, can be used with the Philips PW 9570 flow cell to provide accurate conductivity measurement even when the monitoring electrodes become contaminated. Designed for flow line applications, the PW 9521 automatically compensates for contamination caused by impurities such as oils, fats and emulsions and eliminates polarisation effects, making possible reliable long term measurements with greatly reduced maintenance. When used with a platinum or nickel resistance thermometer, the PW 9521 can be adjusted to provide automatic temperature compensation for a wide variety of solutions with differing temperature characteristics. The PW 9521 can accommodate measurements from 1 ms cm⁻¹ full scale to 1,000 ms cm⁻¹ full scale; the conductivity range being converted to a 0–20 mA or 4–20 mA signal, providing standard inputs to associated monitoring or control systems.

Circle No. 52 on Reader Enquiry Card.

Refrigerated bench top centrifuge. MSE. The Chilspin centrifuge has a refrigeration unit built in and although designed as a bench top model it is easily converted to a floor-standing unit. Chilspin has a maximum speed of 6,100 r.p.m. (6,030g), and four rotors with trunnion carriers and adaptors provide the versatility needed beyond the standard swing-out capacity of 4 × 100 ml, 8 × 50 ml or 16 × 15 ml. Controls include a temperature regulator from +5 to 15 °C, speed indicator, automatic timer and electric brake. Chilspin complies with latest international safety requirements including a positive lid lock, high grade steel guard ring and a hydraulic lid stay.

Circle No. 53 on Reader Enquiry Card.

Column packer. Micromeritics. One of the major recurring costs to the user of liquid chromatographs is column replacement and packing. The Model 705 stirred-slurry column with micro-

particle materials. The pumping unit of any HPLC system can be readily adapted to pressurise the Column Packer which is best used at 6,000 p.s.i. In the 705 the packing material is dispersed in a suitable liquid and then stirred vigorously while being fed into the column. The continuous stirring ensures that a continual homogenous suspension is fed into the column. The technique is simple and fast and it takes about 30 min to pack a 4 mm i.d. × 25 cm column with 10 μm silica gel.

Circle No. 54 on Reader Enquiry Card.



Beckman analyser

Glucose and B.U.N. analysers. Beckman. The Glucose Analyser 2 and BUN Analyser 2 incorporate clinically proven methodologies and advanced circuitry for easier, faster, clinical determination of glucose or blood urea nitrogen. Both instruments are simplified to one-step operation. After calibration the operator injects a 10 μl sample, which automatically initiates analysis. In 15 s the reading in mg dl⁻¹ or mmol l⁻¹ is indicated on the digital display. Three pumps assure fresh reagent in exact measure. A drain pump empties the sample cup, a fill pump refills it with fresh reagent, and a sip pump removes excess fluid until the precise amount remains. Lights on the control panel indicate when to sample and when to read. Instrument chemistries are clinically proven and accepted. The Glucose 2 is based on the oxygen rate method; the BUN 2 uses the conductivity rate method. Both instruments provide answers in 15 s, an advantage in STAT and routine laboratories. Routine output is up to 67 determinations per hour. The 10 μl sample requirement is a further advantage.

Circle No. 55 on Reader Enquiry Card.

Automatic wow and flutter meter. B & K Laboratories Ltd. The 6203 is a small, easy to use but accurate instrument using analogue and digital techniques to measure peak flutter and drift in sound recording and reproduction equipment. It will measure flutter and drift down to 0.001% and 0.01% peak respectively.

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